

What price depleted uranium?

A few weeks before the third review conference of the Non-Proliferation Treaty (NPT), it is disturbing that large quantities of depleted uranium have been sold without safeguards.

A MONTH from now, the signatories of the Non-Proliferation Treaty (NPT) will be gathering at Geneva for a conference to review the terms of the treaty. If this conference follows the two earlier meetings of its kind (1975 and 1980), there will be diplomatic ructions. Even though, by all accounts, preparations have been carried out more smoothly than those for previous conferences, it is probably asking too much of the non-nuclear powers that they do not question the precious little progress that the nuclear powers have made towards their promise under the treaty to negotiate measures of strategic arms control "in good faith". On past form, but also with some reason, non-nuclear powers will argue that more should have been done to spread the civil benefits of nuclear technology. But what will they, and even the nuclear powers which have signed the treaty, make of the puzzling news last week that companies in Luxembourg and Belgium have supplied to Israel several tonnes of depleted uranium in the fissile isotope uranium-235, and that Euratom, Europe's own safeguards authority, was not informed?

It is tempting to think that uranium metal consisting primarily of uranium-238, a waste material in isotope separation plants, has no military significance. Since the first huge hydrogen bomb explosions on the Marshall Islands in the Pacific during the 1950s, it has been clear that ordinary uranium of any isotopic composition may be useful to those who would increase the yield of their explosions. To make a uranium fission-bomb, or a uranium trigger for a hydrogen bomb, it is of course necessary to rely on uranium-235, whence all the fuss about diffusion plants, centrifuges and, now, lasers as a means of separating this isotope from the two others that naturally occur. But in a hydrogen bomb, in which a thermonuclear explosion is ignited by a fission trigger, a surrounding shell of material consisting of heavy atomic nuclei will enhance efficiency by reflecting back into the explosion neutrons that would otherwise escape. Using uranium-238 as the external blanket has the further virtue, if that is the word, that a proportion of the material will undergo fission under the influence of fast neutrons from the explosion. All this was inferred, as long ago as 1955, by Dr Joseph Rotblat using data about the composition of fall-out from the Eniwetok explosion of 1952.

The fact that somebody in Israel has been buying depleted uranium does not, of course, imply that somebody in Israel is interested in making dirty hydrogen bombs. There are many other obvious uses for uranium-238, most of them deriving from its high density and its relative cheapness. But given the trouble that Israel has taken over the years to create the impression that it has gone a long way down the road to the manufacture of nuclear weapons, it is inevitable that suspicions will be aroused. Israel after all, is not a signatory of NPT. Whatever may be the use to which the uranium will be put, however, the fact remains that the sale from the Low Countries to Israel has been made

without the knowledge of Euratom, which has a special status in relation to the international safeguards system in that it has delegated authority from the International Atomic Energy Agency, NPT's inspector, to vouch for what happens within the European Communities to special nuclear materials. The issue has in the past been contentious: why, other members of NPT have asked, should European countries be able to make cosy relationships among themselves without direct invigilation from Vienna? Although it is not quite like that, in that Euratom safeguards are probably at least as demanding as those administered from Vienna, the members of NPT are likely to ask awkward questions about the lapse that came to light last week.

Confidentiality

What should be done? The members of Euratom have a vested interest in demonstrating that their hands are clean, and should do what they can in the next few weeks to show that that is the case. No harm would be done, even after the event, if the companies now found to have been selling depleted uranium on a commercial basis were to be asked, even compelled, by their governments to ask their customers what had become of it. Even if it should be discovered that the customers do not know, or will not say, what is being done with the depleted uranium, that will be useful information, which should of course be made available to the members of NPT. If it should turn out that the users can point to good reasons why they were buyers in the first place, and to the ways in which they are putting the material to good use, that too would be valuable information. The selling companies will no doubt say that such information, being commercial, is also confidential, but that is hardly an argument that should carry much weight; the sellers are, after all, those who have broken the law.

In the past few days, Euratom has clearly been hoping that the sale of the depleted uranium would be quietly forgotten; the names of the selling companies, for example, have not been disclosed. Similarly, no convincing explanation has been given of the source of the material sold, although there are only a few places from which it could have come (the diffusion plants in Britain and France, and the tripartite centrifuge enrichment operation involving Britain, the Netherlands and West Germany in Eurochem). It would be interesting, even significant, to know what Euratom has to say on this question, at the very least because a willingness to abide by the rules on the part of the legitimized uranium enrichment operations involving nuclear powers is an essential precondition of Euratom's chance of continuing to hold delegated authority for safeguarding what happens in Europe.

On the face of things, all this may seem irrelevant to what the NPT review conference will be considering at Geneva — a little local difficulty, so to speak. But that is far from the truth. NPT is already a sufficiently uneasy balance between powerful countries with nuclear weapons and often equally powerful countries that have agreed, for everybody's sake, that they will not go into the business. Transgressions, however small and accidental by the nuclear powers or their agents (those who sell their depleted uranium) could ironically be more unsettling than the errors (mostly of omission) at which the non-nuclear signatories now rail — in particular, the poverty of progress in arms control. □

Biological manuscripts

Contributors are reminded that, with the transfer of the Biological Sciences Editor to the Washington office, it will be helpful if they will in future send **four copies of all manuscripts** offered for publication, either (as at present) to London or Washington.



British politics

Mrs Thatcher asks for decisiveness

Mrs Margaret Thatcher, the British Prime Minister, remains convinced that the British research establishment would have no money problems if it managed its affairs properly. That was one of her opinions on the condition of British science to emerge from an interview last week.

Formally, since the government in 1982 rejected the House of Lords' advice that there should be a part-time science minister on the grounds that it already had a scientist as a prime minister, hers is the desk on which the buck stops. How much time can she spare for it?

Coordinating departments straddling different interests are a mistake, she says, the Cabinet Office in the person of the Chief Scientific Adviser, Sir Robin ("Robin has been wonderful") Nicholson does whatever administrative coordination is needed, and her role is that of a catalyst. She lists the open seminar in September 1983 of scientists and industrialists (with a sprinkling of politicians) of which she was the chairman, and several more recent initiatives. She seems especially pleased about the meeting earlier this year at which £42 million was extracted from the spending departments to assist the growth of numbers of science and technology students in higher education.

One outcome of the 1983 seminar, at which she promised that innovations supported by the public purse would not be offered for exploitation exclusively to the National Research Development Corporation (now part of the British Technology Group) has now been realized. Mrs Thatcher cannot understand why there was so much red tape to clear away. She had wanted individual scientists to benefit more directly from their innovations, but had had to struggle to get her way.

What of the other promise, in 1983, that British defence establishments would be made more open, both to universities and industry? There is some confusion. The defence people have produced an unsatisfactory list of civil spin-off from military research and have been asked to revise it. Fair play, the defence people now have a kind of entrepreneurs' organization of their own.

Wealth creation is the name of the game. Mrs Thatcher says that scientists must appreciate that money for research can come only by the creation of wealth, for which they have a responsibility. But things are not as bad as they seem. She could name a dozen teams of academics pointing in the right direction (and, indeed, she embarks on a list).

On the research councils, she insists that people must be prepared to put their

wishes in some order of priority. Complaints that first-class applications for research grants now often go unsupported are a sign that this is not happening. "People are not prepared to make decisions", she declares.

Mrs Thatcher is also offended at the way research councils administer their affairs. Applicants are asked to include three supporting opinions with their ap-



plications, but then "they send out for another three opinions", which is yet another sign that people are not prepared to take decisions. Why not instead give good institutions a quota of grants and let able professors decide who should get the money? Then the research councils could spend all their administration costs on research.

Reflectively, Mrs Thatcher wonders whether previous governments should not have been more "directional". During the university expansion of the 1960s, people may have been too conscious that the University Grants Committee is independent. The result was that too much of the expansion took place in the social sciences, not science and technology. And where else in the world does the government subsidize business schools?

Now she wants to see more centres of excellence in the universities. The trouble in the past has been that "we've not backed our judgement with money". This is what the University Grants Committee is now aiming at. (Sir Peter Swinnerton-Dyer, its chairman, is also "doing a very good job".) But Mrs Thatcher gives the impression that the recent green paper on higher education will not be her government's proudest achievement.

Mrs Thatcher also thinks it may have been a mistake to leave the judgement of what lines of research would be supported exclusively to the research councils. Earlier (as Secretary of State for Education and Science in the early 1970s), she had supported British high-energy physics. But now she wonders why the research councils are spending so much in this field when the same amount of money would

allow them to do much more elsewhere.

She has little sympathy for the research councils now short of research funds because of having to persuade people to retire early. They have not organized themselves for flexibility. "The worst thing you can do in research" is to put able people in narrow institutes; then "they work on tramlines".

The problem, as she sees it, is that of persuading the research councils to spend their money wisely. They have a "bounden duty" to take responsibility. Now, some people in the research establishment are beginning to do their duty. Sir Henry Chilver (director of the Cranfield Institute of Technology) is one, Dr John Ashworth (vice-chancellor of the University of Salford) is another. Mrs Thatcher contrasts these places, which are "creating jobs" with the other universities, in Manchester and Liverpool, where "we have these huge problems".

On the better exploitation of academic innovations, Mrs Thatcher agrees that industry may in the past have been at fault. People had to hawk their bright ideas around industry, often to no avail. But, she insists, the climate has now changed. It has taken a long time to get managers into a frame of mind to take decisions.

Mrs Thatcher praises the enterprise of the Victorian industrial revolution, acknowledges that one consequence may have been to divert large numbers of bright people into the administration of the old Empire, but says that, now, "the particular combination of genius that we have is not creating the wealth we need. Give me just one MIT", she pleads.

Mrs Thatcher's impatience is understandable, with the money supply once more out of balance. What will happen in the months ahead to the British government's research budget, and the recurrent grant for the universities, can only be a guess. But those who hope for extra funds probably hope in vain.

John Maddox

Informatik stretched

Bonn

THE Westdeutsche Rektorenkonferenz (WRK), official organization of the West German university rectors, has asked for tighter restriction of student access to courses in information sciences (*Informatik*). At their plenary meeting last week, the rectors pointed out that these new courses are overloaded by 200 per cent.

The president of WRK, Theodor Berchem, criticized the decision of the *Länder* (regional governments) to keep these courses open to general access. Part of the trouble is that the number of qualified professors for information sciences in West Germany is too small to cope with the demand. About 50 vacancies have not been filled, and for the planned 4,000 beginners (instead of 2,400 now), 100 additional positions for professors are needed.

Jürgen Neffe

Eureka

France optimistic on meetings

BRITAIN and France are coming closer together on issues of European science and technology policy, to judge by the impressions of M. Hubert Curien, the French Science Minister, of his flying visit to Britain last week.

"I was glad to see we all agree very closely on Eureka" said Curien, after an afternoon with Sir Keith Joseph, British Secretary of State for Education and Science, and Mr Geoffrey Pattie, Minister for Information Technology. Eureka, the French project to develop a series of new high-technology European products from supercomputers to genetically engineered seeds (see *Nature* 11 July p.97), is the subject of a 17-delegation European conference in Paris this week, and Curien was certainly interested during his British visit to test Britain's opinion after British isolation on issues of wider European integration at the recent summit in Milan. He left London "much more optimistic", and undismayed by reports that the British government is interested in Eureka only if it costs no new money. Anyway, Eureka could start with only very meagre cash support, Curien intimated, but would not commit himself to precise figures for fear of prejudicing this week's meeting.

Out of the Paris meeting, Curien is seeking three things. First, an agreement on the principles of Eureka (that should be "no problem"); second, to produce a dossier of the interests of each country in the various "broad fields" of Eureka; and third and most difficult, to define a structure in which Eureka should be managed.

Curien was adamant last week that Eureka should not fall into the embrace of the European Commission. He would like to see a "very light" structure, although he said at a lunchtime meeting at the London-based Policy Studies Institute (PSI) that to avoid bureaucracy altogether could prove "idealistic". The European Commission could not manage Eureka because it had never produced products, and Eureka was strictly product-oriented, he said. The Commission's ESPRIT programme on stimulating cooperation among European companies in pre-competitive research in information technology is a success, but it stops at pre-competitive research — precisely because it is difficult at the Commission to reach unanimous agreement in the European Council (the Commission's political master) on real and potentially competitive products.

Curien is a past president of the French space agency CNES which designed, built and tested Ariane, Europe's space launcher, and naturally he sees the success of Ariane as a model for Eureka. The European Space Agency, which commissioned Ariane, had adopted in 1973 a policy of projects "*à la carte*". Ariane went ahead only because the French wanted it pas-

sionately: they paid two-thirds of the development cost, while Britain stood aside. "If it had been a mandatory programme we would have had no Ariane." Equally, Germany (with Italy) had shouldered most of the burden of developing Spacelab, and Britain had put 50 per cent support into the European Communications Satellite, ECS.

Such projects could not have taken place within the Commission structure, Curien felt, because of the present requirement for unanimity in the Council of Ministers. "The result is always support for the least offensive programme", Curien said, and it is "difficult to be ambitious" within the European Communities structure. (Ironically, the often ambitious Commission officials would certainly agree, faced as they often are by the immovable political obstacle of the Council of Ministers.)

Curien sees the future Eureka management as an "industrial" structure, focused on making things, which should be selected on technical merit and not political representation, Curien insists. And he

agreed with Sir Hermann Bondi, chairman of the PSI meeting, that Eureka will need a technical group "of a quality respected by industry". But "I don't want 17 representatives on everything", one from each state, says Curien.

Asked how Eureka projects would be chosen, Curien emphasized the need for specific, practical projects: "if we just say 'robotics' that would be useless"; but he was less insistent on the need to identify a market for each product. He seemed to be thinking largely along the lines of other major French government-led successes such as the high-speed train and nuclear power, and thus of government purchasers. He admitted, however, that both government and the free market must be considered.

Curien did not expect the Paris political meeting this week to define specific projects — that would be for working groups involving industry later on, once the political ground rules had been settled. However, he did hope to see "five or six" projects under way by the end of this year.

Robert Walgate

The 17 delegations should consist of the 12 European Community countries, plus Norway, Sweden, Austria, Switzerland and a delegation from the European Commission.

US ASAT

Live test tactfully postponed

Washington

A PLANNED July test of the US antisatellite (ASAT) weapon against a live target in space has been delayed yet again, and is unlikely to be rescheduled before Congress renews a moratorium on such tests. The previous moratorium expired in March.

The official explanation for the delay is a technical problem with the instrumented target, designed to simulate the infrared radiation of a Soviet satellite and to monitor the ASAT's performance. The targets have been returned to their manufacturer, Avco Systems, for repairs.

According to congressional staff, however, problems with ASAT itself persist as well. Last December, the Air Force was seeking bids on a contract for the redesign of ASAT's manoeuvring propulsion package, an array of 57 miniature solid-fuel rockets that steers the 12-inch-long, non-explosive projectile into its target. One problem mentioned then was motor exhaust contaminating the infrared sensors that lock onto heat radiation from the target. The failure to resolve these problems is thought to have led the Air Force to delay the test scheduled for July; the ASAT has been returned to its manufacturers, LTV Corporation.

Under current law, the test against a target could proceed if the President certifies to Congress that the test is necessary to avert irrevocable damage to

national security, that it would not damage prospects for negotiating a treaty limiting ASATs, and that the administration is trying to negotiate such a treaty.

The House of Representatives last month passed an amendment to the 1986 defence bill that allows tests only if the Soviet Union tests first a dedicated ASAT system against a target in space. The Senate has adopted a weaker amendment, requiring only presidential certification of his efforts to negotiate a treaty. The US ASAT has so far been fired only at an imaginary target in space.

One suspicion all along has been that the delay in testing against a target, although originally the result of technical problems, has been dragged out for diplomatic reasons.

US officials made it known last week that Soviet negotiators in Geneva have informally proposed dropping their insistence on an end to Star Wars research as a precondition for any new arms-control agreement.

The Soviets are said to be willing to draw a distinction between laboratory research, which could continue, and development and field testing, which would be banned. The Soviet delegates were said to have singled out the US ASAT tests as one example of actions that would not be allowed. The Soviets are expected to submit this new position in a formal proposal shortly.

Stephen Budiansky

UK energy

Department puts money on wind

THE British government, which in 1975 spent £0.5 million on renewable energy research, has since taken a wait-and-see attitude towards unconventional sources of energy. Now, backed by reports from the Advisory Council on Research and Development for Fuel and Power (ACORD), it seems to be coming off the fence. Technologies that the Department of Energy (DOE) calls "winners" — wind, solar, biofuels and geothermal hot dry rocks — will be financed for further research and development, but "less promising" options, notably the £17 million research programme into wave power, will be halted until further notice.

Mr David Hunt, the DOE minister responsible for renewables, earlier this month defended both the amount of government spending and its choices. This year, £14 million has been earmarked for renewables research which, Mr Hunt pointed out, should be increased to £20 million from European and other sources. He claims that these figures demonstrate a commitment to renewables as part of the energy grid.

Questioned whether the increase of cash spending from £16 million in the five years 1975–80 to £68 million in 1980–85 may mask a decrease in commitment, Mr Hunt said that renewables technology "stands up for itself" and should not be judged in terms of the spending on other energy sources. In any case, he claims, the government's programme is "the most positive commitment by any [British] government" to renewable energy sources.

Backing the winners — and wind is "no doubt the most promising" — will mean that more money is channelled into the technologies that ACORD and DOE have labelled as "economically attractive" or "promising but uncertain", and which may make a significant contribution by 2025. Technologies judged less viable, such as converting wave energy into a more usable form, will not be funded but DOE will monitor developments abroad and be ready to change tack. Mr Hunt urged members of the renewable resources community to look beyond their hobbyhorses and to accept the commercial common sense of backing such sources as wind, in which Britain enjoys "world-beating" technology. Only then would these technologies be got "out of the research stations, off the drawing boards and into our daily lives".

ACORD's enthusiasm for wind reflects world optimism, especially in the United States, where wind turbine farms already generate a significant amount of energy. DOE plans to concentrate on large-scale machines, both horizontal and vertical axis, and emphasizes the export possibilities as well as the generation of energy for remote areas of Britain. Research ex-

penditures in 1984–85 of approximately £4.5 million were roughly double those of the previous year; £6 million is budgeted for 1985–86.

The significant British milestones are at Bugar Hill in Orkney and at Carmarthen Bay. At Orkney a 250kW horizontal axis machine, built as a prototype, has operated for 2,303 hours and generated 212,533 kWh; now a 60m diameter 3MW horizontal axis turbine has been approved for the same site. Work at Carmarthen Bay has centred on building a multi-megawatt vertical axis turbine. ACORD estimates for 2025 suggest a 1.6 million tonnes of coal equivalent (Mtce) a year contribution from such onshore sites. Offshore development is still at a theoretical stage, but plans for both national and international projects are afoot.

DOE is also backing passive solar energy, the direct solar heating of buildings, a potential winner that "makes good economic sense". The private sector, according to Hunt, will be encouraged to design buildings that "make the most of sun shine", thereby cutting heating and lighting costs by as much as 30 per cent. ACORD describes passive solar design as a technology that "has come to fruition and is ready to enter the market".

Unlike wind energy projects, in which many technical obstacles impede large-scale use, ACORD finds the impediment to solar energy to be customer diffidence. Potential customers must accept that a solar design does not entail putting up with an unattractive living arrangement. Passive solar's estimated contribution by 2025 is 2 Mtce per year for the domestic sector.

By ACORD's definition, biofuel is non-fossil combustible material, such as dry biomass (straw, wood), wet wastes (animal slurry, food industry wastes) and energy crops (trees or plants grown specifically for energy). ACORD sees organic wastes as the main biofuels in the next 20–30 years. Research is now to be concentrated on the best conversion technologies — direct combustion, thermal processing, anaerobic digestion — for the various forms of waste. ACORD predicts a 1.6 and 8 Mtce a year contribution from wet and dry wastes respectively.

DOE also plans to continue support for geothermal hot dry rocks because of promising experimental results, even though actual output is uncertain. Research is now centred at Rosemanowes, Cornwall, in a project run by the Camborne School of Mines. The idea is to cut holes to depths of 2,000–6,000 metres, artificially fracture the rock and heat water by circulating it through these reservoirs. The project is now waiting for results from a third hole that was recently dug; if these are positive, more and deeper holes will be drilled.

Achieving technical feasibility, however, is not the final hurdle before a renewable energy project is built. These projects often have high start-up costs that are less easily dissociated from the project than are the clean-up costs of conventional energy sources. According to Mr Hunt, allowances will be made for alternative energy sources in Britain. **Elizabeth Collins**

Wind power

West Germany backs out

Hamburg

GROWIAN, (for *Grosse Windenergie Anlage*) the largest wind-driven power plant in the world, is to be dismantled. Situated on the estuary of the Elbe, it could, at least on windy days (around 300 a year), produce 3 MW of electrical power.



Developed during the oil crisis, the windmill was constructed in the early 1980s at a total cost of DM90 million (£22.5 million). Now cracks have appeared in the rotor framework which would require a further DM10 million to repair. Dr Heinz Riesenhuber (CDU), Minister for Research and Technology in the West German government, has decided instead to close the gigantic propeller down next year and to have it dismantled later. The call for the development of alternative sources of energy has become less strident now that the costs of conventional sources have stabilized, which is why the wind energy programme begun by the Social Democrat/Liberal coalition faces its end.

So far this programme has cost DM200 million, a small sum compared with the DM1,600 million invested in nuclear energy, mainly in the fast-breeder reactor plant at Kalkar. The GROWIAN project was at the outset criticized for its enormous size, and for the expected difficulty of operating such a large machine. Its cancellation is seen as a success for the nuclear lobby, which has always maintained that alternative sources of energy have no future in West Germany. **Jürgen Neffe**

French biotechnology

Making high-tech champagne

BIOTECHNOLOGY is about to be applied to those distinctively French wines and cheeses. That is the novel component of the recently approved biotechnology programme of the ministry of research and technology.

The French government and the agro-food manufacturers, France's second largest industrial community, are worried that their traditional methods are too unreliable, variable and sensitive to uncontrollable factors such as the weather to secure their place in the world markets against high-technology producers in California, South Africa and Australia. So the French food producers have decided they will use science to help.

Champagne will be one beneficiary. One of the most difficult elements of champagne technology, *rémuage*, is the removal of spent yeast from the wine after the second fermentation that produces the "fizz" and the flavour. This is already being changed by the use of "fixed", pelleted yeast. Moët-Hennessy, the producer of Moët et Chandon and many other well-known champagnes, is carefully introducing the new methods.

The company says there is no detectable effect on the flavour, and that the advantage will be that *rémuage* will take only three days, compared with the present laborious three months of slowly turning and raising the bottles until they are cork-down, followed by a swift opening and closing of the bottle by an expert to blow out the unwanted yeast. With fixed yeast, the same blowing-out is necessary, but the yeast pellets sink far more rapidly than individual yeast cells.

The saving of storage time will increase by 80 per cent the capacity of the champagne cellars that burrow throughout the champagne district, says Moët-Hennessy, which would help iron out the immense fluctuations in champagne production caused by the weather. (Champagne, one of the world's most northerly wine-growing areas, produced 300 million bottles in 1983, but only 80 million in 1981.)

The vines themselves are also under study. The small Programme Nationale de la Vigne, established three years ago by the research ministry, held its first scientific conference last spring and raised hopes that gene cloning and transfer techniques together with cell fusion and tissue culture might well produce useful new varieties. In the past, it has taken between 20 years and a century to make a new clone adapted to a particular soil and climate; this time must be shortened if new varieties resistant to disease or less sensitive to spring frosts, for example, are to be developed.

Cheese production, usually by small local makers, is also in question in a dairy industry which was shocked to hear re-

cently that Japan, which is on a wave of fashion for French cuisine, has begun to manufacture its own high-tech but tasty Camembert. This apparently uses French organisms but Australian milk, and thus represents a large market which the small French producers have almost totally lost. Scientifically, the questions again are how to achieve constancy, quality and volume.

At the ministry, the view is that after three years of government aid, biotechnology is already well established in the large French drugs, vaccines, bio-reactants, fine chemicals and medical manufacturers, but that the small agrobusinesses now need a helping hand. Many of the companies are too small to have any research effort at all. Hence, according to Pierre Douzou, director of the new gov-

ernment *programme mobilisateur* for biotechnology, seven out of ten of the industrial advisers for the programme will be drawn from the agrofood industries, and the emphasis of the new programme, supported by FF 115 million (£11 million) of seed money for 1986, will be in agriculture and food.

The new trend has also been emphasized by Douzou's further appointment as chairman of the scientific council of INRA, the national agricultural research organization, which has "many fine scientists" but has been historically somewhat isolated from other French scientific communities, particularly those supported by the national and medical research councils, CNRS and INSERM, and even from the universities.

Douzou sees his job as helping INRA director Jacques Poly to "opening up" INRA to the rest of science.

Robert Walgate

European professions

Plan to make diplomas travel

Brussels

THE European Commission has drafted legislation laying down the principle of mutual recognition of all higher education diplomas throughout the Community. The aim is to facilitate the mobility of those exercising a profession. The European Commission seems to think this can be achieved without harmonization of national higher education systems. But the draft directive is very much at the beginning of a consultative process in which the European Parliament, industry, employers and various lobby groups, as well as governments, will want their say.

The measure aims at tying up a rather large loose end in Community law, whereby it is illegal to discriminate on the grounds of nationality, so that Community citizens have the right to set up their professional domicile in any of the ten countries of the European Economic Community (EEC), but where paradoxically only nationally acquired qualifications are recognized in order to be able to exercise one's profession. The only exceptions are doctors, veterinarians, dentists and architects, for whom mutual recognition of diplomas already exists.

But whereas in these cases academic curricula were to some extent harmonized throughout the Community as well, this time the Edonnino committee, mandated by heads of state at Fontainebleau in June 1984, recommended, instead, just an overall system of mutual recognition and trust, rather than the time-consuming harmonization of training courses.

The draft directive covers all professions requiring three years of higher (university-type) education which have followed a higher secondary course — *baccalaureat*, *abitur* or A-level.

It thus applies equally to paramedics

(physiotherapists, opticians, psychologists), the scientific community (engineers, geologists, agronomists), lawyers, journalists, economists, statisticians and teachers.

Information on professional recognition would be available from a computerized network linking up centres that already exist.

While the proposal is clearly an expression of political will on the part of governments towards the European ideal of complete mobility of citizens, making it easier for those whose home market is saturated to move to another EEC country, in practice it may come up against the protectionism and corporatism that characterized some of those professions.

Secondly, it is clear, as the proposal points out, that despite some measure of similarity or comparability, differences between the education and training systems in a given profession will still require the newcomer to obtain further experience or training in the proposed country of establishment. The proposal allows for a maximum period of three years but makes no budgetary provision at Community level, nor does it specify who will pay. Under Community law, a directive lays down the aims but leaves the means of achieving them to the member states. This could give rise to different financial constraints in different member states.

Achieving some level of uniformity between national education systems could further enhance the mobility aimed at by the draft legislation, but there is no provision for harmonizing education in the Rome treaties. Even if EEC education ministers do meet to discuss equal opportunities for students, this is an area in which they jealously guard their sovereignty.

Anna Lubinska

Polish universities

Doubts about concessions

POLISH universities seen at last to have won concessions from the government in their campaign against the proposed reform of the 1982 Higher Education Act. According to the leading Warsaw weekly, *Polityka*, the government Committee for Social and Political Affairs, in charge of drafting the reform, has given way on five major points. But many academics fear that these concessions are essentially cosmetic, designed to allay further, last-minute criticism of the amendments which, it is believed, will go before the Sejm (Parliament) in the near future.

According to *Polityka*, the Committee for Social and Political Affairs has decided:

- to drop proposals to replace tenure for senior lecturers and professors and to replace it by a contract system;
- to drop the proposal that rectors, pro-rectors and deans should be appointed by the Minister of Higher Education and Science instead of being elected by the universities themselves;
- to retain elections to the Main Council for Higher Education, the 70-person body representing all Polish universities and higher education schools (except the private Catholic University of Lublin);
- to allow universities to decide their own statutes instead of having to conform to a standard form of statutes dictated by the Minister;
- to retain some form of student "self-government committees", instead of replacing them by bodies linked to party youth organizations.

Although, formally, there was a major public debate on the proposed changes earlier this year, the *Polityka* article is the first open admission that the universities (as represented by the Main Council) are diametrically opposed to the government over the changes.

Moreover, *Polityka* cited several leading academics who contradict the assertion of the Minister of Science and Higher Education, Dr Benson Miskiewicz, that falling academic performance over the past three years is clear proof that the 1982 act has been used to "destabilize" the universities. The rectors of Warsaw Polytechnic University and the Jagellonian University of Kraków, in particular, are quoted as saying that while, unfortunately, there has been a decline both in the percentage of students completing their courses and in the quality of their final dissertations, there is considerable doubt as to whether this is a result of the 1982 act. This apparent "openness" of the *Polityka* article has given some encouragement to the more optimistic academics.

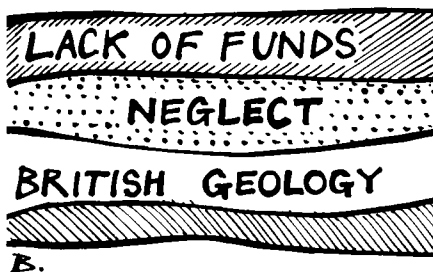
On the other hand, the less optimistic point out, there was never any real likelihood that tenure would be abolished.

Even a tenured lecturer can be dismissed by special decision of the Council of State (as happened recently in the case of Dr Leszek Nowak, a social scientist at the University of Poznań) and the proposal was probably included in the draft as a bargaining counter. The election of university officials will be carried out by a senate which will consist almost entirely of senior tenured staff, and without the participation of representatives of the student body and non-teaching academic employees. The abolition of elections to the Main Council is said to have been proposed, as a result of the vehement opposition shown by that body to the changes, as a means of reducing its power. Although it will not be changed into a puppet body of ministerial appointees, the Main Council still loses its decision-making powers, so that, under the reform, the minister will have only to "consult" the Main Council on terms of academic employment.

British geology

Crying in the wilderness

THE saga of the declining budget for British geology took a new turn last week. In the most public and formal *cri de coeur* from the geological community so far, nine presidents and chairmen of major professional bodies* published a tract in which they summarize the present economic circumstances of their disciplines and the dire consequences they anticipate if fundamental changes, including an increased proportion of mineral taxes to be



spent on research, are not soon made.

The decline in research commissioned by government departments, as well as cuts in funds from the Natural Environment Research Council (NERC) has led to a virtual halt in strategic surveying by the British Geological Survey (BGS) (see *Nature* 311, 499; 1984). The new document, sent to Secretary of State for Education and Science Sir Keith Joseph last week, summarizes this position but puts it in the most general economic terms. In 1983, the total mineral production (including oil) by Britain was worth £24,000 million; the tax and royalty payments from North Sea oil alone in 1984 amounted to £9,000 million. Funds from NERC for the geological sciences in 1983–

Even more serious, the minister will still retain what many see as the greatest threat of the reform, the right to dismiss or suspend any university official or lecturer, expel or suspend any student, and close down or suspend any department, without appeal or independent disciplinary hearing, if he deems it "in the public interest".

Furthermore, one should read not only *Polityka*, which still enjoys "by inertia" something of its pre-1981 reputation for a liberal outlook (by Polish standards) but also the Party daily *Trybuna Ludu*, which, only a few days previously, had given its own views of the reform. According to *Trybuna Ludu*, opposition to the reform is virtually equivalent to opposition to the Party, and, it implied, under the amended act, university employment would be dependent on an oath of loyalty to the concept of a socialist university. The idea of such an "oath" came as a surprise to most academics, who throughout the whole campaign have been largely dependent on "leaks" to the Solidarity underground press for information about what the government was planning. **Vera Rich**

84 amounted to 0.0043 per cent of the latter figure.

The signatories of the tract go on to outline the "impasse" with which they are confronted. In answer to the question of whether academic and institutional research is worth saving, they say, "the common government response is that if industry wants it, industry will pay for it. Industry usually replies that, as they have already paid for it in taxes and royalties, government should pay." The government's way out, say the signatories, is to tell the universities to fund research from consultancy service, an approach which, while being increasingly adopted, is bringing universities into conflict with the private sector.

The solution proposed is, in the short term, an injection of £15 million "earmarked specifically for the geological sciences... adequately to fund systematic surveys, to computerize national data-banks, to re-equip research centres of excellence and to allow UK participation in major international projects". And NERC? The document uses weasel words: "The role of NERC needs to be evaluated and the rationality of its Corporate Plan assessed". **Philip Campbell**

* Signatories: P.Allen (chairman, British National Committee for Geology), C.H.Holland (president, Geological Society), D.C.Ion (president, Institution of Geologists), W.G.Yuill (president, Institution of Mining and Mineralogy), J.Birks (president, Institute of Petroleum), P.I.Allsop (president, Institution of Mining Engineers), M.G.Audley-Charles (chairman, Committee of Heads of University Geology Departments), R.F.P.Hardman (chairman, Petroleum Exploration Society of Great Britain) and J.R.Vail (chairman, Committee of Heads of Polytechnic Geology Departments).

Nuclear insurance

US Congress prepares new rules

Washington

AN obscure decennial ritual that will determine who pays in the case of a nuclear accident is about to begin again. The Price-Anderson Act, which sets a limit on the financial liability of nuclear utilities, expires next year; lobbying by industry and its opponents is already shifting into high gear.

The act, first passed in 1957, was designed to remove a major obstacle to the adoption of nuclear power by electric utilities: the risk of a crippling financial burden in the event of a catastrophic nuclear accident. The act removes the right of injured parties to sue anyone but the utility, and places a limit on the liability of utilities. (The figure depends on the number of licensed reactors in the country; it is currently \$630 million.)

In its original form the act provided for the federal government to play the role of chief insurer. That provision was altered when the bill came up for renewal in 1967 and 1977 and each utility is now required to carry \$160 million in insurance; in addition, if an accident occurs that causes damage in excess of that amount, each reactor will be assessed up to \$5 million — a sort of after-the-fact insurance premium. The federal government is now liable only for accidents at its own facilities, and only up to \$500 million.

Industry, in pressing for a renewal of the act, argues that it is not a subsidy to the nuclear power industry, but rather a form of "no-fault" insurance, akin to worker's compensation. Without the liability limit, industry argues, victims of an accident might receive even less — judgements of more than a few hundred million dollars would bankrupt the average utility.

Critics point out, simply, that a major accident could easily cause damage to the extent of tens of thousands of millions of dollars; they claim that by shielding the industry from this risk, the act skews economic incentives to increase safety.

Opposition is also emerging from states that are candidates to receive federally operated depositories for high-level reactor waste, as the act limits the amount that victims of an accident at such a facility can recover to \$500 million.

Even if Congress fails to renew the act, however, the reactors already licensed would probably continue to enjoy the protection of the act. US courts have long held that legislation such as the Price-Anderson Act represents a financial contract that cannot be undone by subsequent legislation. At issue is whether new reactors coming on line after 1 August 1987 will be covered.

Several bills have already been filed in Congress:

●Simpson-McClure (S 1225) would maintain the basic framework of the act while

increasing the liability limit to \$2,000 million.

●Morrison (HR 2524) would provide for full compensation for any accident involving high-level waste.

●Hart (S 445) would repeal the liability limit while retaining the current insurance scheme; victims would be free to sue any liable party if damages exceed \$630 million.

Another alternative, endorsed by the Nuclear Regulatory Commission, would provide full compensation to victims by assessing after-the-fact premiums from all reactors at \$5 million a year for as long as it takes.

Stephen Budiansky

Genetic engineering

Rifkin set back

Washington

THE US District Court of the District of Columbia has denied the latest attempt by anti-genetic-engineering activist Jeremy Rifkin to put legal obstacles in the way of a deliberate release experiment. Judge Aubrey Robinson ruled that the National Institutes of Health (NIH) could not, as requested by Rifkin, be compelled to regulate a proposed field test of a genetically modified bacterium intended to protect crops against frost damage. The experiment, proposed by Advanced Genetic Sciences Inc., has now been submitted instead to the Environmental Protection Agency (EPA).

The experiment was recommended for approval in June 1984 by NIH's Recombinant DNA Advisory Committee (RAC), but the director of NIH withheld his approval to avoid applying different standards to private companies, as opposed to academic researchers: a previous injunction forbade NIH to approve an almost identical experiment by Stephen Lindow of the University of California until NIH prepared an environmental impact statement.

Dismayed by the legal circus surrounding the Lindow proposal and the prospect of long delays, Advanced Genetic Sciences withdrew its application (which was in any case only voluntary) and went to EPA, which has claimed legal authority over deliberate releases of some recombinant organisms.

Rifkin petitioned the court to rule RAC's original recommendation of the Advanced Genetic Sciences experiment invalid, on the basis of obscure legal technicalities; he then sought to compel NIH to forbid it.

In dismissing both petitions, Judge Robinson made plain his puzzlement with Rifkin's argument, and the experiment now lies firmly in the lap of EPA.

Tim Beardsley

Leprosy vaccine

Malawi trial to be launched

ALTHOUGH more money has yet to be found, the British Leprosy Relief Association (LEPRA) has announced the launch of a full-scale trial of a newly developed leprosy vaccine in northern Malawi in 1986 in conjunction with the government of Malawi, the London School of Hygiene and Tropical Medicine (LSHTM) and the World Health Organization (WHO).

About 15 million people suffer from leprosy, and although drug treatment is available, it can be very slow and often too late to prevent irreversible neural damage. Excitement is understandable at the development of a potential vaccine, which has been made possible largely by the collaborative efforts of a group of scientists within the WHO Special Programme for Research and Training in Tropical Diseases (TDR) coordinated by Dr Barry Bloom of Albert Einstein College of Medicine, New York.

The vaccine consists of heat-killed *Mycobacterium leprae*, the causative agent of the disease, purified from the tissues of the nine-banded armadillo, the best animal host for growing large numbers of bacteria which cannot be grown *in vitro*. Four colonies of armadillos are maintained by TDR for this purpose. Safety trials have been completed in Norway, where leprosy was eradicated as recently as 1950, and a large-scale non-random trial of 60,000 leprosy contacts is under way in Venezuela which will be completed later this year.

The Karonga district of Malawi has been chosen for the first population-wide trial largely because prevalence of the disease is rather high (1–2 per cent) and because of the groundwork laid by LEPRA's comprehensive evaluation programme in this region directed by Dr J.M. Ponninghaus and coordinated by Dr P. Fine of LSHTM. Over the past six years, at a cost of approximately \$500,000, all 120,000 residents of the region have been examined and interviewed, and improved serological tests have been developed. About 1,700 leprosy cases are present and up to 240 new ones have appeared each year.

There is evidence from earlier trials that vaccination with Bacille Calmette-Guérin (BCG) against tuberculosis can provide some protection against leprosy and, indeed, animal experiments and therapeutic work in Venezuela suggest a vaccine containing both killed *M. leprae* and BCG is most promising. The aim therefore is essentially to compare this mixed vaccine with BCG alone in a randomized control trial of the whole population except those who do not wish to be involved and those who will be excluded on the grounds of age, ill-health or already diagnosed as leprosy cases.

Nigel Williams

Creationism down under

SIR—Tony Thulborn is to be congratulated on his exposure of the Creation Science Foundation in Queensland (*Nature* 9 May, p.89) and will be pleased to know that the Australian APE (Australian Association for the Protection of Evolution) is not entirely alone.

The Institute of Biology in Australia (IBA), which is a branch of the Institute of Biology and on the road to total independence, has already challenged creationists in a major symposium, "In defence of science — A response to creationism", held in Sydney with an audience of more than 200 people on 9 March. The meeting was sponsored by IBA jointly with the Linnean Society of New South Wales, the Royal Zoological Society of New South Wales and the Australian Museum Society. Speakers successfully countered persistent (and ill-informed) questioning by creationists, some of whom freely admitted to teaching creationism unofficially in science classes at all levels, in schools in New South Wales, thus confirming Thulborn's suspicions.

The proceedings were videotaped by staff from the Commonwealth (Australian) Department of Education. A short film will be released to schools soon. The papers and discussion (edited by Dr Frank Burrows) will be published by the University of New South Wales as the first volume of a new series entitled *Australian Studies in Biology*.

The fight against creationism will be continued at the second annual general meeting of the institute in Brisbane on 5–7 July. A motion will be put affirming *inter alia* that creationism is not a science and thus has no place in any biology syllabus in Australian schools.

JOHN SKIDMORE

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SIR—Michael Cavanaugh's implications in his article "Scientific creationism and rationality" (*Nature* 16 May, p.185) cast some doubts as to whether creationism can muster sufficient support from *bona fide* academics and especially from PhDs holding established positions in reputable scientific organizations. One of the main reasons for this state of affairs, of course, is that creationists are threatened by their own colleagues and by legal action by their own school boards and institutions if they do not keep a strictly low profile either publicly or in their own class rooms. Fear of loss of respect and prestige, not to mention endangering their own jobs, is what keeps highly qualified creationists in their ivory towers (even to the extent where they avoid open association with outspoken creationists). I speak from person-

al experience and from the knowledge of what other creationists are going through.

C. K. PALLAGHY

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SIR—Having reread *Genesis* for the first time in several years, prompted by Cavanaugh (*Nature* 315, 185; 1985) on creationism, I still fail to see the difference between creationism and evolutionism apart from the time-scale, which could easily be resolved by postulating that one theological day corresponds to a large number of millennia, in which case we are still waiting for the events of the eighth day. If we accept this approximation, then the only difference between evolutionism and creationism is the question of the catalyst.

As the hypothesis of the creationists is a supernatural force whose existence can be established only in the after-life, it would seem reasonable to withhold judgement until after death when the hypothesis will be confirmed or refuted. Given the many millennia which modern evolutionists would consider to correspond to a single creationist's day and the relatively short time since the creation of man, which marked the end of the sixth day and which was followed by a day of rest, it would seem likely that death for most of us alive today will be an earlier event than the creationist's eighth day.

Further speculation is futile, providing creationists will concede to the evolutionists that the theological day is indeed in excess of 24 worldly hours.

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Jobs in Spain

SIR—Recently, because of the large number of technical and academic professionals emigrating during the Franco years, King Juan Carlos let it be known that no Spaniard should ever again feel that he (or she) could not work in Spain. Unfortunately, the inertia of 40 years of scorning knowledge and its human heritage leaves the royal wish unheeded in the spheres which could do something about it.

During the Franco era and even afterwards, a small but steady stream of young Spaniards left Spain to do research or to benefit from higher education abroad. Many now wish to return. The national propaganda (words and wishes) wants them back; the system, however, is not willing. In Spain, positions at universities or research institutes are awarded by peculiar and primitive ritualized examinations called "oposiciones". In principle (and on paper), they are open to any Spa-

niard. The reality is different; only Spaniards with a Spanish degree can apply. In short, a degree of any kind from a foreign university (regardless of its name or place) is not valid and the knowledge that accompanies it is questioned. Of course, the bureaucracy provides a simple way out: "convalidacion" (validation). One can apply to validate one's degree; what this involves varies and, in general, is inversely proportional to how many influential personalities the applicant knows.

It is a sad and deplorable situation, particularly in the euphoria of Spanish integration in Europe. The absurdity of the situation is clear from the fact that any Spaniard with a Spanish PhD can obtain a job in Europe or the United States, without any bureaucracy or any more questions than those related to the job; any Spaniard with a PhD, from, say a US, English or German university has to fall into a "windmill battle" with Spanish academic authorities and thus is not eligible for a job in his/her own country.

It is important that these ancestral prejudices disappear. They are probably a consequence of the isolationism that has characterized the Iberian peninsula for centuries. The accession of Spain to the European Community attempts to break the barriers that extend above the Pyrenees. Let us hope that it breaks all barriers. ALFONSO MARTINEZ-ARIAS
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Scientist arrested

SIR—We have heard from scientists in Poland that their colleague, Zbigniew Gluza, has been arrested. Gluza, aged 33, was employed at the editorial board of the journal *Informatics*—the leading publication in the field in Poland—and is a member of the Polish Informatical Society. He was also a member of Solidarity.

Zbigniew Gluza has become a victim of the regime and an example of the planned programme of repression being suffered by Polish intellectuals.

During a random inspection in the street in Warsaw, the Polish *milicja* found some popular underground literature in Gluza's car. It should be pointed out that these underground bulletins play an important role in Poland, because official publications do not carry a genuine news digest—only officially edited reports which are usually distorted by ideology and its powerful instrument, censorship.

Letters of protest are being written to Polish embassies, consulates, cultural and scientific institutions. It is to be hoped that international groups and societies will add their weight to the protest. The efforts of private individuals will also be welcomed.

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Science at a very great distance

Australia and New Zealand are in a different part of the world from most other places, as the cover shows. They are also a long way from each other, geographically and in other ways. This survey of science in the two countries is necessarily far from complete. It is offered as evidence of the vitality and promise of what is happening in these interesting places.

Australia's technical revolution

THE government of Australia is in danger of talking itself into a muddle about science and technology. Australia is blessed not only with great natural resources and a splendid climate but with a superb scientific enterprise. The government has rightly diagnosed the economic decline of the past few years as the consequence of Australia's failure to increase exports of manufactured goods to compensate for the diminished value of exports of primary materials, agricultural produce and minerals, itself a consequence both of the world-wide recession and, in agriculture, of protectionism (as by the European Communities). For this reason but also by the inspiration of Mr Barry Jones, the Minister for Science, Mr Bob Hawke's Labor government has been, during its two years in office, embarked on a great drive to turn Australia into a technological community. It is a brave and laudable ambition, which should have enlivened the technical community. Why, then, are the people who work in academic and research laboratories more anxious, even despondent, than they have been for many years?

A large part of the trouble is that the technical community, while sharing the government's aspirations that technology should be made economically more valuable, fears that the government will not put its money where its mouth is and that its enthusiasm will get the better of its judgement. Last year's failure in the budget process to make a substantial increase in the funds of the meagrely supported research grants scheme for academics is worrying, as is the requirement that the public research organization (CSIRO) should do more (industrial innovation, especially) with less. And there is little sign that the Hawke government's conviction that the supply of skilled people from Australian universities must be increased will be followed by commensurate support. Will the government show that it means what it says in next month's budget?

Exorcising anxiety of the second kind will be more difficult. The prophet of the revolution the government hopes to work, Mr Barry Jones, is politically more radical than pragmatic Hawke, but he properly

appreciates that basic science is as crucial a part of technological change as is the engineering of innovation. The practitioners of the revolution are harder men who share Jones's vision and impatience but not his understanding. Collectively, they give the impression that scientists who are not working for industry are a bunch of layabouts. The obvious danger is that they will intemperately enforce changes that damage Australian science without yielding much in the way of economic benefit. Aesop's fable about the dog, and the reflection of his bone in the water, applies.

The torrent of public introspection since 1983 about the engineering of Australia's revolution has been more confusing than clarifying. This month's second draft of the "National Technology Plan" (from which Mr Jones mildly dissents) is even fuller than last year's version of specific goals, and actions to be taken by government and others. Even in a country as used to being overgoverned as Australia, the proposals seem over-dirigiste; is it possible, let alone desirable, that part of the government's subsidy of universities should be "earmarked" for "mission-oriented basic and applied research" intended to make existing industries more competitive and to create entirely novel industries? That is what the plan says.

A more serious weakness of the public argument about the future is that it seems innocent of serious consideration of what Australia might be like economically if and when the revolution has come to pass. Australia seems ready to turn its back on the traditional sources of its wealth. In the dash for a new prosperity, and with the knowledge of what the foreign exchanges have been saying this past year about the dangers of over-spending, the government's motto seems to be: "If people don't want our beef, we'll do research on something else". And so, inevitably, there is a new strategy for research, with priority objectives clearly spelled out: biotechnology, information, robotics and the application of computer-aided design to manufacturing technology and materials. Australia, with its 15 million people, is bursting to follow every European country in attempting to simulate what has been happening in the United States and

Kiwi alert

NEW Zealand's scientists face hard times. The government has decided progressively to lower the budgets of the major research organizations over the next three fiscal years. There will be further cuts in real funds, particularly in the universities, as inflation, now running at 15 per cent, makes itself felt. For the government, the falling budgets are regarded not so much as "cuts" as incentives for the recovery of funds from elsewhere. The gap that opens up as grants are reduced is meant to be filled by increasing the amount of money taken in from industry and from other government departments by contracts and by the sale of services. In a nutshell, "the user must pay for science".

In as far as they are to suffer financial austerities, scientists find themselves in much the same boat as many others in New Zealand. Despite its celebrated anti-nuclear stand, the new Labour government has brought in tough monetarist policies. It inherited a massive deficit from the outgoing National Party government and decided that a sharp shock was needed to wake New Zealand to the harsh economic realities. The first action of the new Prime Minister, David Lange, after winning the election last year was to devalue the dollar by twenty per cent; subsequently, it was allowed to float and its value has fallen further. Import licensing systems which had long protected local manufacturers were done away with, along with controls on financial markets. Some farm subsidies were also thrown out. Next, there is to be radical overhaul of the tax system aimed at slashing personal taxes and passing the bill to goods and services.

It is not hard to see why Lange decided on drastic action. In the mid-1950s, the country was the third richest in the world measured by per capita income. Now it is close to twentieth. The cause of the decline can be summed up in a sentence: the balance between primary and secondary industries is not appropriate to keep a small country in prosperity. In New Zealand, primary industry means agricultural products, principally meat, wool and dairy, making up 80 per cent of its exports. Markets for them are becoming harder to find and more and more unstable.

The solution to the problem is, in one

AUSTRALIA and New Zealand, until recently among the most prosperous countries in the world, have fallen on hard times. In each case, the explanation is that world demand for primary products has fallen. Each country has responded by setting its sights on the exploitation of science and technology to make products that will find new markets. For what it is worth, both countries have also within the past two years elected governments from the left of their political spectra, Mr David Lange's Labour government in New Zealand and Mr Bob Hawke's Labor government in Australia. (The word "programme" is also spelt with a single "m" in Australia.)

This survey of science in both countries is necessarily incomplete. *Nature* wishes to thank the many scientists and politicians in both countries for their helpful courtesy and also Dr Jeffrey Sellar, *Nature's* Canberra correspondent, and his predecessor, Dr Vimala Sarma.

NOTE: \$A1 = £0.51; \$NZ1 = £0.35

Japan. Nobody seems to have thought much about the possibility that when demand for the traditional products is depressed may be the worst time to rob them of research support.

Yet what is wrong with Australian industry is not that Australians are unenterprising, or that Australian scientists are unimaginative or even that Australian workers are lazy — these are the opposites of the truth — but that Australia created for itself in the comfortable 1960s and 1970s an unreal economic framework whose abandonment not even the most radical politicians can advocate (in public). Australia has indulged xenophobic instincts about capital investment (wholly-owned subsidiaries of overseas companies are bad) and chauvinistic instincts about overseas trade (high-cost local manufactures behind tariff barriers are preferable to cheap imports). The result is that Australian manufacturing industry is inward-looking, persuaded that its only worthwhile market is Australia. So it is no surprise that Australian imports of technologically-based products are worth eight times the corresponding exports, or that the volume of research carried out by Australian companies is tiny. (For Australian companies, the domestic market is not big enough to support full-blooded research; overseas companies do their research at home, although ICI and Kodak are exceptions.) Yet Australia's determination to become a technological economy has been prompted by the recent discovery of this unsurprising state of affairs.

This is why Australia could best satisfy its ambition to become a technological community by liberalizing the framework which has so far held it back. Mr Hawke and his Treasurer, Mr Paul Keating, have spent the past few weeks battling for a radical reform of domestic taxation

(which is a good cause, but one in which they will only partially succeed). To make their technical revolution happen, they must also reform the external economic relations of Australia. Devices such as the 150 per cent tax allowance (see p.189) will no doubt help, but can be no more an assurance of success than is the palliation of symptoms a cure for a disease.

What the government must appreciate is that, with 15 million people, it cannot be (and does not need to be) a powerful source of innovation and production across the whole field which it now wishes to explore. Indeed, Australia would be on safer ground if it were to build more energetically on the strengths it has already. The application of biotechnology in agriculture (animals as well as plants) is full of a promise that has been recognized. The reasons why Australia ships metal ores and the coal with which to reduce them to countries overseas (Japan particularly) is a puzzle, but one in economics. Australia's apparently perverse determination not to add economic value to the uranium it sells abroad is political in origin, but a costly self-encumbrance. Australia's medicine has and deserves the highest reputation internationally; where are the pharmaceutical industries that would have sprung up elsewhere?

The risk in the indiscriminating course the government seems bent on following is that the quality of the present scientific enterprise will be undermined. That is what alarms many academic scientists. A modern state such as Australia needs to be in touch with what is happening in the whole field of science (which is why there should be no shame that many CSIRO laboratories are able to hold a watching brief on what may be happening elsewhere). There is no harm in offering people incentives to see their bright ideas turned into saleable products, although it is absurd that there should be such pressure from the government to ensure that Australian innovations are exploited only by Australian companies. But to expect that every working scientist should contribute directly to the revolution is a recipe for disaster.

The government's greatest need is for patience. It is right to look for a technological revolution in Australia, but not to expect this to happen overnight. It does not much help that it has chosen this time to review the operation of pretty well every important organization spending money on science. Might not some of them be allowed to get on with what they do already? (CSIRO, the largest public research organization, is in a different case; its management structure is not appropriate for the functions now expected of it; see p.189). Although part of the trouble is the short interval between elections (never more than three years), the government must learn to recognize the excellence of the science enterprise it has inherited and learn to meddle less. □

sense, obvious. First, value must be added to New Zealand exports: more pulp and paper and less timber, more carpets and less wool. Second, the range of products must be increased; new types of cheese must be developed, new fruits grown, and meat processed in new ways for new markets. And third, more manufactured goods must be developed in areas where New Zealand is "believable"; farm equipment, software packages for farm management, portable communications equipment and the like.

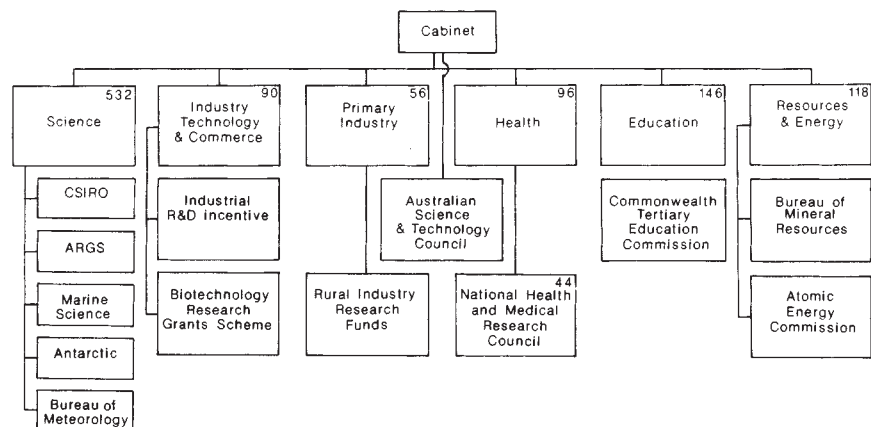
How to bring about these changes is less than obvious. Lange's policies have so far proved a major shock to the economy. Growth has fallen to less than one per cent this year compared with four per cent last year. But in a phrase that will be familiar at least in Britain, Lange says that New Zealand cannot continue to "live beyond its means".

The bludgeon that has been applied to the economy is now to be applied to science. The government may yet regret what it is doing. New Zealand's problem in research is that there is a great deal of talent, of which a large part lacks the means to show what it is capable of.

The imaginative technological changes needed in the economy require that talent be put to use. But simply trying to harness science to industry through contract work is unlikely to be productive, for industry does not have a clear enough idea of its own long-term interests to be able to direct research sensibly. Nor does it understand that without basic research before long there will be no applied research worthy of the name. The government pays lip service to the idea that science must be promoted. It seems not to see the danger in reducing budgets indiscriminately.

There may be an explanation. All the signs are that the government is remarkably ignorant of science and technology and generally holds scientists, as against managers, in low regard. Occasionally one hears that the "Japanese way" should be followed; and that all science should be put in the service of industry. But that is not the real situation in Japan. If anything distinguishes that country, it is the premium placed on being well-informed about scientific matters: the Prime Minister heads the key scientific policy-making body and there are reams of reports and statistics. In contrast, it was only a few months ago that anyone in New Zealand had more than a half-accurate idea of how much industry was spending on research. What the government should be doing is finding out what the nation's scientists — and the universities constitute a major pool of under-used expertise — can contribute. And that identifies what seems to be a major weakness of the present administrative system. No powerful voice represents science to a high level of government. But without giving science high priority, how can the technological change on which New Zealand has set its mark ever be achieved? □

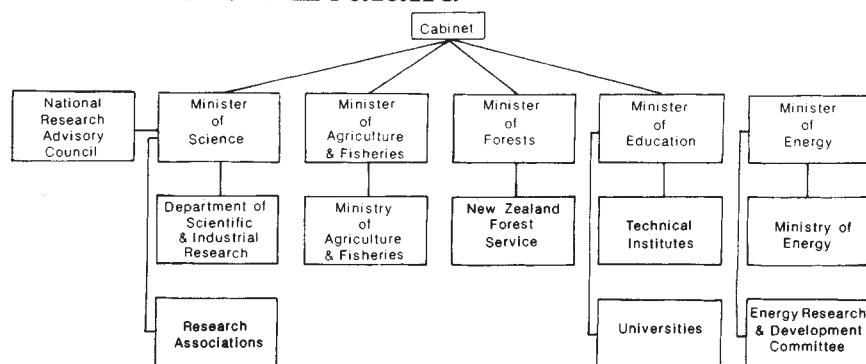
How science is run in Australia...



Most government ministries spend some money on research. (Defence is excluded from the diagram above.) The numbers attached to each ministry are the estimated expenditures in 1984-85 on research and development in millions of Australian dollars. The particular complication of Au-

stralia is that the federal structure shown above is paralleled by a comparable pattern of administration at the state level; especially in agriculture, states are substantial spenders on their own account (while research funds are also recruited by means of a levy on certain products).

... and New Zealand



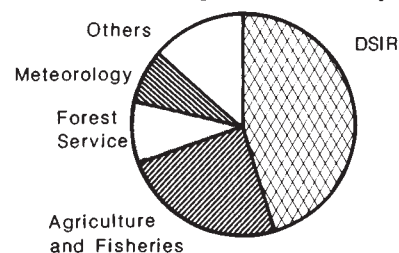
As well as those shown above, two other ministries have research arms: Transport (New Zealand Meteorological Service), and Works and Development (Water and Soil Research and Survey group). Grants

are also provided by the Medical Research Council to universities, hospitals and its own small research units for dental research, virus toxicology, immunopathology and alcohol research.

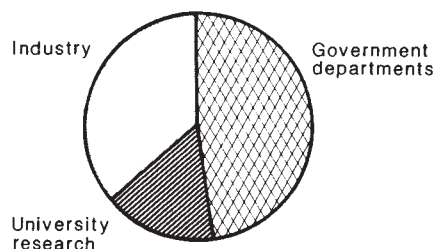
Science and technology



FUNDING for New Zealand's research and development for the year 1984-85. Government spending is \$NZ150 million, the universities \$NZ200 million and industry \$NZ69 million. Had the chart been drawn a little earlier it would have been divided into quite different slices, for it has only just been discovered that industry spends a considerable sum on research. There is a simple reason for that: because expenditures on research for new products (as against current product lines) are not tax-deductible, research costs have consistently been hidden under "maintenance costs" and other such categories. A new survey has revealed the above figures and in doing so destroyed a recent OECD analysis which centred on the belief that industrial research and developed effort was tiny.



THE Department of Scientific and Industrial Research (DSIR) takes the major share of the government funds.



Texture of diversity in Australian science

THAT Australian science is in good shape needs only the most cursory inspection. Traditionally, agriculture is the country's strong suit; growing crops in arid regions is one of the exportable technologies developed, veterinary medicine another. Since 1945, biomedical research has flourished, largely at the urging of Lord Florey and Sir Macfarlane Burnet. So too has astronomy, partly because of Australia's geographical position in the Southern Hemisphere.

What follows is a kaleidoscope of impressions gathered on a rushed journey during June. By being selective, it is necessarily unfair except in the sense that the random citation of a few high-quality projects may prove that there is much more of the same unmentioned.

Australia is strong in the application of *in vitro* fertilization to childbirth. Dr Carl Wood, professor of obstetrics and

gynaecology at Monash University, and Dr Alan Trounson, direct one of the centres. Wood says that his work is a natural extension of veterinary developments in Australia. He reckons that throughout Australia, there are 10,000 women waiting for treatment, more than 2,000 at his own clinic.

Melbourne is a medical powerhouse, with two important medical schools and a traditional backing of outstanding public hospitals. The university has also become one of the several centres of biotechnology in Australia, since when its botany department has been singled out as a centre of excellence by the Tertiary Education Commission. Dr Adrienne Clarke, head of the centre, says she decided deliberately to spend half of her \$A2 million grant on mass spectrometry equipment. Partly as a consequence, her laboratory has become even more the hub of collaboration on the

molecular basis of plant cell physiology.

Throughout Australia, specialized institutes such as the Hall and John Curtin institutes and university departments are turning as quickly as they can to molecular biology. So is CSIRO, partly on the strength of a report by a committee under Dr N.K. Boardman in 1979.

CSIRO's Division of Molecular Biology in the North Ryde suburb of Sydney is one of the smaller divisions, with a professional staff of 48, roughly one-third of them paid for by contracts with outside organizations. How does such a laboratory cover the ground?

Dr G.W. Grigg, the division chief, is a little alarmed that too many of his bright people will devote themselves exclusively to practical projects, making the laboratory less effective at its indispensable role as an impartial source of advice for Australian industry at large. In practice, the

laboratory has already helped to spawn a substantial company, BIOCLONE, an international supplier of monoclonal antibodies specific for pituitary hormones.

Sydney houses a clutch of important CSIRO divisions, including that for mineral physics which claims to have pioneered remote sensing in Australia. Among other things, the division is using magnetotelluric techniques for mapping oil reservoirs, and claims the first systematic application of palaeomagnetism to the study of ore bodies. But its practical product is an instrument called SIROASH which uses nucleonic techniques to measure the bulk ash content of coal; the underlying phenomena are gamma-ray scattering and electron-pair production.

Sydney is also one of the centres of Australian astronomy, largely because of the long collaboration between the department of physics at the university and the CSIRO Division of Radiophysics. Sydney physicists are the prime movers in the plan to build the Australia Telescope, a \$A25 million radio interferometer consisting of an array of six 25-m dishes strung out along a track 6 km long.

This ambitious project is the responsibility of the CSIRO Division of Radiophysics, which believes it will be able to engineer an instrument whose frequency coverage, accuracy and sensitivity will be without precedent. The radiophysics division hopes that its expertise in the design of antennae feeds will contribute to the success of the equipment.

Optical astronomy is also prominent, the flatness of the ancient continent of Australia notwithstanding. The Mt Stromlo Observatory (40 minutes from Canberra), part of the research school of physics at ANU, claims to be the one of the most influential sources of professional astronomers in the world. Its facilities are remarkable, but mostly to be found 500 km to the north, at Siding Spring, which is also the site of the Anglo-Australian telescope.

The latest acquisition is a home-designed 2.3m telescope built at a cost of \$A3.2 million and opened in March 1984. Dr Don Mathewson, director of Mt Stromlo, is especially proud of three features of the project — the fact that the six-inch thick mirror blank (thin as these things go) was fashioned by sawing a Corning blank in half laterally, the altazimuth mounting of the telescope and the fact that the building in which it is housed rotates as a whole on its axis.

The Anglo-Australian Telescope is similar kettle of fish. Dr Dan Morton, the director, reckons that Australian astronomers profit more than British from the facility. As of last month, nobody at the office had been informed officially of the decision by the British Science and Engineering Research Council that continued support (£1 million a year by each side) did not come high on the research council's list of priorities. People in Sydney

say that the agreement to operate the telescope is embodied in a formal bilateral treaty between the governments concerned, and that it cannot be abrogated by the agent of one of them. The next few months should be interesting.

Australia's interest in astronomy has also in the past few years been furthered by the plan, now aborted, for a tripartite collaboration (with the United States and Canada) to operate a 1-m reusable telescope package from a low orbit about the Earth. In Canberra, they have no doubt that the system would have been more economical than the Hubble Telescope.

So Mt Stromlo is now hoping that something will come of another plan, involving both the US National Aeronautics and Space Administration and the European

Space Agency to launch a far ultraviolet detector as part of the Fuse/Columbus project.

This is the conventional part of the exploration of the world outside the Solar System; the radical part is the experiment, based on the physics department at the University of Sydney, by means of which an attempt is being made to put flesh on the bones of a proposal due to Professor R. Hanbury Brown to build large-scale Michelson interferometers from which to extract the diameters of distant stars and galaxies. The department is now bringing into commission an interferometer with a baseline of 11.4 m. Then it will set out to raise more than \$A2 million. Either way, like much else that happens in Australia, the instrument will be unique. □

ASTEC

Vehicle for wise council

THE most remarkable component of the Australian government's machinery for the administration of science and technology is the Australian Science and Technology Council (ASTEC), a device by which independent scientists not only give advice when asked but have a kind of prescriptive right to comment on government policies with technical implications. The closest analogue of ASTEC is historical — the US President's Science Advisory Council (PSAC) in its heyday of the 1950s. PSAC, alas, had lost much of its influence by the time it was abolished in 1971; ASTEC, on the other hand, was invented less than a decade ago and was given a statutory place in the machinery.

ASTEC is not a substitute for a Minister for Science; there is one of those, Mr Barry Jones. Nor is it the minister's creature; rather, it reports directly to the Prime Minister, now Mr Bob Hawke. The council itself, now eighteen strong, is a roughly equal mixture of academics and users, mostly from industry. The chairman is, Professor R. O. Slatyer, director of the School of Biological Sciences at the Australian National University.

Unlike science advisory committees elsewhere, ASTEC is adequately staffed. The secretary, Dr B.D. Middleton, emphasizes that ASTEC's distinctive role is its oversight of the whole of government business (for which reason the organization has access to cabinet papers). Often, he says, ASTEC has persuaded the government that a proposal given only low priority by a minister should be given more serious attention. ASTEC claims some responsibility for the decision a few years ago to re-equip the Bureau of Meteorology and to contribute to the international seismic network for the surveillance of nuclear explosions.

An important part of the philosophy of ASTEC is that its members work hard (they are now paid \$A11,000 a year). Last month, they were collectively finishing off

a round of visits to all the 40 divisions of CSIRO as part of a major enquiry into the role of research in Australia.

By definition, ASTEC cannot avoid being part of the political process. So much is clear from the review of Australian uranium policy, published a year ago (see p.200). One explicit objective of the inquiry was to consider the adequacy of safeguards on the export of uranium.

It seems not to have damaged ASTEC's standing that the government was unable to accept the key recommendation that Australia should play a fuller part in the fuel cycle than as a uranium supplier.

ASTEC seems also to be capable of political initiatives. The government's announcement (in May) that from the beginning of the next financial year (1 April 1986), expenditure on research and development by private companies will qualify to the tune of 150 per cent as a business expense had its origins in ASTEC's glooming about the low level of expenditure by Australian companies.

The present inquiry about the function of research in Australia is a different kind of nettle. The Hawke government's determination that almost every public institution should be reassessed suggests that the question would inevitably have had to be grasped at some stage. That CSIRO should be singled out for the first and most contentious chapter of this crucial examination suggests either that ASTEC has woodenly accepted terms of reference that prejudice the outcome, or that ASTEC has preconceived ideas about the location of buried bodies. The best guess is that the truth is neither extreme.

None of this is a complaint. In the past year or so, ASTEC has tackled subjects as different as the place of Videotex in Australia and technology's role for the handicapped. Part of the reason for its influence is that it will deliver what ministers (of whatever party) need. Now and again, it may be able to stir the pot. □

Government's role

Minister with his own vision



In a system of parliamentary government, in which the officers of the executive are chosen from among the people's representatives, every government needs one minister like Australia's Minister for Science, Mr Barry Jones. Whether it could live with two like him is another matter.

Mr Jones, less of a minister than a prophet with political pretensions, is known to both those who have never met him and his enemies (who abound) as "Barry". Barry, in the argot, rendered Prime Minister Bob Hawke a signal service before and after the election of March 1983. Single-handedly, he has made science and technology into an issue that ordinary people take seriously. Nowhere else, except possibly in India now and the Soviet Union in the 1930s, has the exploitation of science and technology been as proper a subject of polite conversation as it has become in Australia.

Mr Jones first became a national figure as an astonishingly successful competitor in the television quiz shows of the 1960s. People still tell of the occasion when he proved that the television network and not he had given the wrong answer. Long memories, of course, do not necessarily make for congenial politics.

Mr Jones has a sense of duty and is considered to be on the left-wing of the Labor Party. He is the author of *Sleepers Wake!*, a tract which, in 1983, sought to persuade Australia that it must use and live with technology if it is to hold its own.

Mr Jones has kept talking about the need for change consistently since taking office in March 1983. In the process, he has popularized the problem of technological change and also helped to confirm his own popularity. But there have been two substantial set-backs. The first, just a year ago, was a shock to those of Mr Jones's supporters who may have thought he was about to wave a magic wand; in the event, the funds for providing university research grants (see p. 194) did not increase by the promised 10 per cent but only by the amount of inflation.

Explanations of what went wrong differ. Some say that the minister brought the trouble on himself by putting increased science spending a long way down his list of priorities, certainly after his proposal for the Commission for the Future (not another exercise in futurology but an attempt to increase Australia's capacity for change). Some think he was playing a devious game by counting on the government's commitment to ensure that the increase came through. The most likely explanation, however, is that the meagreness of the increase may have been a reprisal for Mr Jones having fought within the Labor caucus for complete withdrawal from the uranium mining industry.

Mr Jones's second setback came only after the general election last December, when the Hawke government was returned with a reduced majority. The technology part of the science and technology portfolio was whipped away, and handed to Senator John Button, previously at the industry and commerce ministry, one of the government's strong men and (unlike

Mr Jones) also a member of the Cabinet.

The reasons for the change seem to have been administrative rather than political. Just as he is not cast in the standard ministerial mould, so he is by everybody's consent except his own a poor administrator. Priorities change too often: he creates a sense of rush when civil servants prefer serenity. Serenity is not Mr Jones strong suit. Much of his personal popularity stems directly from his open friendliness. He is almost too open to be a successful politician, and in any case, he is too imaginative. □

CSIRO's industrial might

INDUSTRY has always been an important component of CSIRO's terms of reference. The novelty is Australia's new-found conviction that it can no longer rely on agriculture and minerals to balance the cost of the products its people must import. CSIRO seems eager to play its part.

So the organization now has a fully-owned commercial subsidiary to help with the exploitation of innovations arising in the divisions and called SIROTECH. (SIRO is CSIRO's way of referring to its role as originator in projects as different as SIRONET, a computer network, and SIROSMELT, a small-scale but efficient way of extracting base metals from ores.)

After only 18 months, it is too soon to know how effective the new company will be. Its terms of reference allow it to become the manager of intellectual property, patents and the like, arising from research at the divisions. The company can also invest in "pre-development" work.

Meanwhile, division chiefs seem still to have much of the responsibility for the exploitation of their people's work. Dr G.W. Grigg at the molecular biology division at Sydney reckons to have been the one who

persuaded a Sydney businessman to invest in the production and marketing of a range of monoclonal antibodies. Dr J.R. Anderson at the materials science division at Melbourne worries on a day-to-day basis about the zirconia ceramics project.

Except in agriculture and minerals, Australia has traditionally been a protectionist economy. One consequence is that the Australian manufacturing sector is neither export-conscious nor compelled, by the international market, to be as conscious as would be wise of quality.

So innovators are faced with a dilemma: should they license their bright new idea to an Australian company, knowing that they can help guide the development of the product through the critical stages and earn a substantial royalty on sales at the end, or should they sell the licence to some company overseas, earning a smaller percentage on what may be a larger volume of sales?

Hitherto, political considerations (not to mention the character of CSIRO) have forced local licensing policies on the organization. Will SIROTECH break this pattern? □

CSIRO

Time for thinking again



FOR much of the period since the Second World War, the name for Australian science has been CSIRO, short for Commonwealth Scientific and Industrial Research Organization. During most of that time, the organization has ridden high, rightly boasting of its splendid achievements in agricultural research, innovative work in basic research and of being the chief custodian of Australia's international reputation in research.

But now the gilt is flaking off the gingerbread. The universities are stronger than they used to be, while government is increasingly concerned to know what benefits it gets for all the money (\$A382 million in 1983) and the services of all those people (more than 7,000 staff, over 2,000 of them professionals). So CSIRO is on the defensive. ASTEC (see p.188) has been asked to inquire into the entire role of the research enterprise in Australia, but to give priority to its views on

CSIRO, which is conscious of having blotted its copybook with the new government and which is responding a little frenetically to the clamour that research should benefit manufacturing industry.

Some in the government do not forgive CSIRO for the letter sent by its chairman Dr Paul Wild to the incoming government asking that the organization should be transferred from the Department of Science and Technology; the letter went off before the cabinet had been sworn in, and so was delivered to the out-going Fraser government, irritating both camps.

But even those with the shortest memories recall the great row over the Australian National Animal Health Laboratory at Geelong, near Melbourne, partly because of the high cost (\$A157 million) but mostly because the plan to import live foot-and-mouth disease virus had not been discussed with a quarantine-conscious agricultural community.

Farmyard computers and biotechnology

At the CSIRO Division of Plant Industry it is clearly passé to ask what impact molecular biology is having on agriculture. People have stopped thinking of it as a subject; it is rather a set of tools which everyone uses.

There is no doubt that the division is a dynamic one and that it has a dynamic boss in its director Jim Peacock. One suspects that a man who became director of a plant industry laboratory by working on *Drosophila* will have no difficulty in persuading government of the value of whatever he feels should be done.

Balance between basic research, "strategic mission-oriented research" and short-term applied research is maintained at 20:20:60 per cent. But there is clearly a premium placed on generating new concepts for agriculture from basic research (such as the developments of diagnostic kits for plant viruses) rather than just "agricultural improvement".

It is not only molecular biology that is taken for granted in the division; computers have also arrived. One hundred million dollars worth of the annual cotton crop is now grown to a tune sung by a computer-

based management system, SIRATAC.

In the SIRATAC system, years of data collected on crop growth, insect pests and insecticide effectiveness are built in. Every few years the farmer inputs on his own terminal the numbers of insects on his cotton and the numbers of fruit; the central computer adds weather data. The program then predicts from the likely effects of weather, beneficial insects and previous sprayings the mortality of fruit and the number likely to survive to harvest. This is not so simple as more fruit are produced than will mature; light insect damage might even increase yields by removing unnecessary fruit.

Beyond SIRATAC come systems for other types of irrigated agriculture. SIRACROP is now poised for commercial development and will decide irrigation schedules for farmers growing wheat. And beyond that again comes a total system for a range of crops — soybean, maize and the like — which will not only increase farmers' profits but also stop the poorly managed use of water contributing to rising water tables.

Alun Anderson

One casualty of this distrust seems to be Dr Wild, the chairman, whose term of office ends in September, and who will be replaced for nine months by Dr Keith Boardman, formally due to retire as a full-time member of the executive of CSIRO on the same date. Dr Wild, previously director of the Division of Radiophysics at Sydney, and the driving force behind the distinctive Australian system for landing aircraft automatically, is generally thought to have been poorly equipped with the mixture of guile and directness required for success in Canberra. CSIRO is unusual among the descendants of the organizations set up in the 1920s in what the British call the old Commonwealth. Its terms of reference cover everything from agriculture to minerals extraction and metal processing to general support for manufacturing industry. Two years ago, CSIRO adopted a self-denying ordinance on medical research, agreeing that it would not undertake clinical research except in collaborations.

CSIRO's structure is as unusual as its scope is unbounded. The the chief executive is the chairman, appointed by Canberra, who has two full-time fellow members of what is called the executive, on which day-to-day decisions fall. There are also five part-time members, whose function seems to resemble that of the non-executive members of corporate boards — to act as informed referees. Key decisions rest with this handful of people. There is also an advisory council whose function is not so much to participate in decisions as to interject opinions.

Part of the more obvious trouble is that this band of men, housed in a new piece of modern concrete half-way to the airport at

Canberra, is necessarily a long way from where research is practised. Day-to-day matters take precedence: thinking ahead is harder. The remoteness of the executive has been enhanced by an earlier reorganization, in 1972, when individual laboratories (called divisions or sometimes units) were grouped into five bundles, called institutes, each with a director of its own. These, too, appear to be condemned to too great a burden of paper-pushing. The real directors of research are the heads of the divisions (of which there are 44), known as "chiefs" and addressed by their colleagues by that word. Typically, a chief will be a man in his fifties, often one with scientific distinction overseas, but who will usually volunteer that his chief function is to provide an environment in which younger people can be creative.

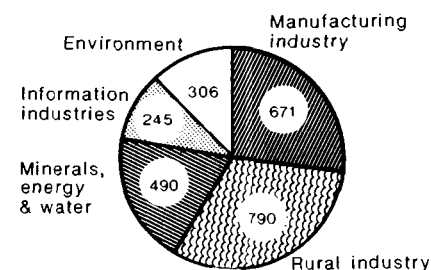
Scale is more difficult. Typically, a division will have about 50 working scientists, formed into perhaps half a dozen research groups. Some division chiefs admit that their laboratories are big enough only to make a few distinctive contributions to the international enterprise so as to earn the right to keep a watching brief on what is happening elsewhere.

The quality of the work seems nevertheless to be uniformly excellent. So why do Australian governments not cherish CSIRO as in the old days? Part of the explanation is that there has been a change of government. Although discontent has been rumbling for many years (witness the 1972 reorganization), and while the Hawke Labor administration is in no sense a doctrinaire socialist government, it was elected partly on the strength of Mr Barry Jones's plea that Australia should prepare to join the twenty-first

century (see p.188); it was natural that CSIRO should have felt threatened by its arrival.

Some of the accusations made against CSIRO are nevertheless unfair. Thus it is widely held that the organization is not, or not sufficiently, accountable. Unlike many other research organizations, however, CSIRO is not autonomous. Quite apart from the familiar mechanism of control by annual budget, CSIRO's enabling act (of 1949) gives the government the right to issue directions to CSIRO. Internally, CSIRO operates a system of periodic reviews of its divisions, the consequences of which are often the amalgamation or reorganization of laboratories, or substantial changes of the emphasis. If accountability at the bench means a sense of people looking over one's shoulder, CSIRO is blameless.

CSIRO is, however, vulnerable to the complaint that it is ingrown. Unusually, it is a career research organization. During the past quarter of a century the supply of home-grown research scientists has grown from a trickle to near-sufficiency. CSIRO has become a substantial source of academic and industrial scientists, but its core staff has become over-used to what many think of as the traditions of the service. Bringing in people from outside, especially to senior posts, could not fail to help.



CSIRO's research effort by purpose and/or beneficiary, 1983-84. Numbers indicate professional scientists engaged. Source: CSIRO Annual Report.

CSIRO is also large, in Australia, conspicuously so. The central administration of close on 50 establishments and the wise arrangement of objectives in some coherent order would tax the most enlightened organization. The concentration of responsibility on the shoulders of three full-time executives seems to predetermine both the appearance of stubbornness and that of irresolute compliance to outside pressure.

The terms of reference are in some ways an encumbrance: "... to plan and carry out a comprehensive program of research on behalf of the Commonwealth for the benefit of the people and industry of Australia". Research policy is thus not easily distinguished from public fashion.

The present policy (see *Annual Report, 1983/84*) is to transfer to seven fields (biotechnology, advanced materials, manufacturing technologies, information technology, water and soils, plant pathology

and oceanography resources that can be quarried from elsewhere by natural wastage (3.5 per cent a year).

The recent growth of numbers of those working in these fields is impressive evidence of how a determined executive can make things happen; the danger is that the interest of continuing research in both minerals processing and, more particularly, agriculture, will be undervalued. The importance attached to biotechnology may have agricultural implications, but there seems to have been only scant consideration of the continuing need for research in agriculture, or even that when markets turn sour, the need for research may be all the greater.

The other side of this coin is that CSIRO may have less to offer industry than it believes. Historically, CSIRO's specific terms of reference have included responsibility for "strategic research" in support of technology. It is also the undisputed case that many divisions have been the source of valuable industrial innovations, and that Australian companies are too neglectful of the value of research and development. But while there is no distinctive economic pattern in Australian manufacturing industry, at least from the standpoint of the export markets, it is hard to see that CSIRO's efforts to reinvigorate the manufacturing sector can do more than spread resources thinly over unknown territory.

Certainly there are many Australian industrialists who complain that CSIRO should not harbour ambitions in this field. Thus Dr Lou Davies, research director of the electronics and defence manufacturer AWA, has publicly said that CSIRO should stay in the patch it knows. Among the division chiefs, who seem to accept the new policy willingly, there seems some confusion about the degree to which basic research, whatever its potential value to manufacturing industry, is permissible in the new regime.

These difficulties could be enormously simplified. Outsiders are bound to ask why a felt need to improve the research base of manufacturing industry should require the retreat from agriculture and minerals. The questions would be more easily decided if there were three distinct organizations for agriculture, minerals and manufacturing industry.

Dismemberment is the least preferred solution at CSIRO, which is natural enough. Fair play, there are other steps that might be taken to make the organization less vulnerable, among which a revision of CSIRO's management structure seems the most obvious.

Over the years, the troika which is the executive has become the day-to-day management; the influence of the part-time members varies, but often requires that able scientists should be involved in the choice of priorities for the years ahead (at which they are not necessarily skilled). It could not but help if this group of people

functioned more like a board of directors of a company, not (as they sometimes appear) a rubber-stamp.

It is hard to think that in such an environment, universities would continue to occupy as marginal a place as they do at present in CSIRO's thinking. The days (in the 1950s) have long since gone when universities were dwarfed in competence by CSIRO. Yet CSIRO does not yet behave as if its remit "to plan and carry out a comprehensive programme of research . . ." could be read as an invitation to place major research grants within university departments. The benefits would

be flexibility, perhaps even lower costs but also the further blurring of the old patronizing relationship between the professional researchers and the teachers.

None of this is to suggest that CSIRO is indifferent to university problems. The physics department of the University of Sydney has succeeded largely because of its past collaboration with the Division of Radiophysics, while the planned Australian Telescope will be a great asset for university groups throughout Australia. But by being more guileful, might not CSIRO also be more effective, and have more friends?

John Maddox

Walter & Eliza Hall Institute

Towards a malaria vaccine



THERE seems no stopping the Gus Nossal show, the Walter and Eliza Hall Institute of Medical Research at Melbourne. At the end of last month, the institute was given by the government one of its largest ever grants — more than \$A6 million over five years for its contribution to the development of a vaccine against malaria. In keeping with the spirit of the times, the government's Industrial Development Board will have a right to 40 per cent of the value of the intellectual property that emerges.

That the Hall institute, technically an independent charity, should win a commanding place in Australian biomedical science is largely the doing of two successive directors, Sir Macfarlane Burnet (1944–65), a 1960 Nobel prizewinner, and now Sir Gus Nossal (since 1965). But being in Melbourne helps. Melbourne medicine, concerned though it may be with the curing of the sick, is also anxious to dominate Sydney, and so is warmly supportive of its own institutions.

Burnet, at 85, is as chirpy as ever. An immunologist before molecular biology came of age, he gave the Hall institute intellectual standing at a time when Australian science was regarded as derivative of that in the United Kingdom. He denies that he has dragged his feet on molecular biology, in his book *Genes, Dreams and Reality* (1971), but argues the need that researchers should be patient. His small-talk (of which there is not much) tends to cosmology as much as immunology.

Nossal is more ebullient, much more the leader (and driver) of men. Austrian by birth, and a protégé of Burnet, almost all his professional life has been spent in Australia. (There were two postdoctoral years at Stanford.) The Hall institute is still small enough (with a staff of 200) for Nossal to continue to function as director of the cellular immunology unit at the institute, one of the eight between which the institute's work is divided.

The most tangible sign of the institute's success is the new building, financed

largely by the governments of Australia and Victoria, into which Nossal hopes to move later this year. One of the institute's frustrations is that the building is at least a year behind schedule, largely because of labour troubles during construction. Nossal says that some features of the design were incorporated into the building at Cambridge, Massachusetts, in which the Whitehead Institute is now housed; he finds it galling that a building on which \$A20 million has already been spent should be so far behind.

The move to the new building, 100 metres away, has been made necessary by the growth of the past few years, and by the need of space in which the institute is now housed. Until the latest grant was announced, the malaria vaccine programme accounted for roughly a quarter of the whole programme, whose cancer research unit has now become a Ludwig institute.

Nossal's operating costs are low by US standards but higher than in the United Kingdom. Last year, the institute spent \$A7.3 million, of which more than a half (\$A3.7 million) came from the federal government's grant-making agencies, chiefly the National Health and Medical Research Council. The Victoria government contributes \$A500,000 a year, while Nossal is proud last year to have pushed the value of grants and contracts from overseas above \$A1 million for the first time (with the help of the Rockefeller Foundation and the US National Institutes of Health).

The malaria vaccine programme is inevitably the most glamorous part of the institute's programme, although Nossal says (before the latest grant) that it accounts for only a quarter of the total effort. In reality, the present excitement is the product of ten years of development at the Hall institute, and malaria (particularly that caused by *Plasmodium falciparum*) is only one of the half-dozen or so parasitic diseases in which the unit has an interest.

The present objective, based on a

knowledge of the genome of *P. falciparum*, is to develop recombinant antigens characteristic of the parasites that may be expected to stimulate the immune response when injected into people. The key development (see *Nature* **306**, 751–756; 1983) was the demonstration that antigens characteristic of the blood-stage (merozoite) parasites can be expressed in *Escherichia coli* and that surface antigen, itself a molecule with relative molecular mass of 200,000, contains a repeating unit of eleven amino acids. Since then, the parasitology group has been able to show that peptides derived from naturally occurring *P. falciparum* proteins and containing even shorter repeating sequences of amino acids can react with immune sera (*Nature*, **310**, 789; 1984).

So the way seems clear for a systematic programme of work with a high chance that it will lead to the means of immunizing people against malaria parasites carrying the particular antigen investigated. Robin Anders, head of the malaria team at the Hall institute, is nevertheless eager to emphasize the difficulties ahead. The extent of the variability of the antigens between the different strains of just *falciparum* is not yet settled, while the task of working through the genomes of the other species of *Plasmodium* responsible for human malaria has hardly begun.

Another difficulty ahead is that an effective human molecular vaccine will almost certainly have to stimulate immunity against at least two other antigenically distinct forms of the parasite, that carried in the liver and the sexual stages transmitted to the mosquito vector. As a consequence, Dr G. F. Mitchell, head of the immunoparasitology unit at the Hall institute, says that there is now close collaboration with other groups working on these stages, the Nussenzweigs at New York University for example.

Even before the \$A 6 million grant from the Australian government was announced, Nossal was optimistic about what lies ahead. He reckons that it should be possible to tell what the vaccine cocktail should contain and to have laboratory evidence of its efficacy within three years, and that a working vaccine may be used for the first time in five years or so. Nossal is also full of praise for the WHO Special Programme of Research and Training in Tropical Diseases, whose existence coincides with that of the Hall institute's explicit interest in parasitology.

For the rest, the laboratory is up to its neck in cell biology of all kinds. Nossal says there is virtue, nowadays, "in being able to call almost anything immunology". Nossal's own group is engaged on the hunt for the chemical messengers between lymphocytes which are presumed to modulate the immune response, generically the lymphokines; one of its more recent successes has been the characterization (down to amino-acid sequence, in

collaboration with Dr Lee Hood's group in California) of the P-cell stimulating factor (PSF), thought to stimulate the proliferation of haematopoietic cells when released by activated T cells.

On the principle that immunology spans all cell biology, the Hall institute is inevitably involved with questions such as the maturation of T cells in the thymus. (The local doctrine is that only a minority of

thymocytes eventually survive as T cells.) The translocation of the *myc* gene in oncogenesis is just as inevitably prominent in the research programme, but the promise for the future may lie in the programme of the molecular biology unit, which is concentrating on the relationship between oncogenes and growth factors (and the receptors for them) in the causation of cancer. □

Universities

Entangled in red tape

THE universities of Australia are in a state of flux, but that is nothing new. Since the 1940s there has been an almost continuous examination of the function and operation of educational institutions, both universities and the Colleges of Advanced Education (CAE), many of which have evolved from the old state-run teachers' training and agricultural colleges.

The consequences of this selfconsciousness have been beneficent. The system has grown, and is still growing. Among the nineteen universities proper, there is an interesting diversity, neatly illustrated by the differences between, say, the James Cook University of North Queensland (p.195) and the University of Adelaide (p.198). Since the Second World War, the universities as such have also been transformed from undergraduate teaching institutions to institutions with substantial and effective research programmes and graduate schools. So why is there persistent anxiety about the working of the system?

The arrival of the Hawke government in March 1983, with its commitment to more open access, was inevitably a spur to further introspection. There are three sets of issues in the air — budgetary problems (including the question of whether students should be required to pay fees, as used to be the case before the 1974 Whitlam government), support for the direction of research and the management of the system as a whole.

The pursuit of administrative tidiness over the past decade in particular has complicated the management problem. Professor David Caro, vice-chancellor of the University of Melbourne, has said that the administration of higher education in Australia "is the most complex in the world... no other country ... has invented such an involved co-ordinating and advisory apparatus".

This is how it works. The Australian universities (nineteen plus the Australian National University at Canberra) are supervised by the Universities' Council, whose existence goes back to the recommendation of the Murray committee in 1956 that Australia should interpose a council similar to the British University Grants Committee between government and the universities; the complication, in

Australia, is that there are two tiers of government, federal and state. Since then, two further higher education councils have emerged, one for CAEs called the Advanced Education Council and one for Technical and Further Education (TAFE). The notion that each of these councils should make separate claims on federal and state government funds was recognized by 1974 to be a recipe for conflict, which the Whitlam government sought to rationalize by creating a Commission for Tertiary Education to coordinate the work of all three councils. The commission came into being only in 1977; the delay in part is explained by the dissolution of the Whitlam government by the governor-general, Sir John Kerr, in 1975.

What now seems clear is that even the new system is unworkable. The newly appointed chairman of the Commonwealth Tertiary Education Commission (CTEC), Mr Hugh R. Hudson, puts the point eloquently in a document published earlier this year, a response to the federal government's request for yet another review of the administration of higher education.

Before 1974, the federal government contributed only 35 per cent of the public subsidy of higher education, the remainder coming from the states, but the Whitlam government (with the agreement of the state governments) took financial responsibility to itself in 1974. The result, according to Hudson, is that the states support "the sometimes unrealistic claims of the institutions" in their territory. He says that in the bidding for funds for the three-year period beginning this year, one university, apparently with the support of its state minister of education, asked for a capital sum of \$A100 million, close on two-thirds of total federal spending on capital development in higher education during the previous three years.

Administratively, the present system is also cumbersome. Each council is expected to produce a coordinated bid for federal funds which is then coordinated into a unified submission by CTC. In effect, Hudson says, much of the work is done twice, the volume of paper delivered to the government tends to confuse by its bulk and contradictions and, in any case,



CTEC's supposedly coordinated advice has often to be submitted before the three subsidiary councils have finished their own documents.

Hudson's complaints will be generally accepted but his proposed solutions are controversial. Hudson would turn all three subsidiary councils into advisory committees, concentrating responsibility in his own CTEC; both the universities and the state-oriented TAFE sector are against the proposed change, although the CAEs appear to think they would gain either from that or from an amalgamation of their council with that representing the universities.

Hudson is less clear in his proposals for changing the federal/state relationship. He asks that states should determine their own priorities for the relative development of the three sectors, which is sensible enough; he also says that CTEC should itself have close relationships with universities and CAEs, which need not prejudice the present sponsorship of universities and CAEs by the state governments; but he shrinks from considering whether there should be some change in the present division of financial responsibility.

Two related themes run through Hudson's ambition for the system — the need that socially disadvantaged groups of young people (immigrants, called "migrants", and aborigines) should have opportunities in higher education, and the need for greater cooperation between the three sectors. The first objective is widely accepted, by the government no less than by individual institutions. The second will raise more serious difficulties, even though there are already several institutions that span the boundaries; Hudson points out that James Cook University offers courses appropriate for CAEs, and that the Royal Melbourne College of Technology provides teacher training as well as TAFE courses. But Hudson is also looking for arrangements under which credit can be given for courses which can then be transferred elsewhere.

Hudson's plan would also transfer to CTEC responsibility for ARGS (p.194). The argument is that there is now no assurance that those winning grants have the resources to use them effectively, that the result would be greater efficiency and economy, and that CTEC could better assess the need for an increase in ARGS funds than would the science department.

The Hudson prescription for administrative reform is certain to be influential; the government will have to respond to it before the end of the year. But the plan to take over ARGS, one of Hudson's admittedly "more distant" goals, is also thought to be his own negotiating ploy, something that can be given away later on. The more serious question hardly ever raised is whether institutions, and universities in particular, might be liberated from the sense of detailed remote control from which they now suffer. □

Students and budgets hit plateau

THE growth of Australian universities over the past decade has been dramatic but has now stagnated, as have the budgets of individual universities. Many now argue that, without saying as much, the federal government is requiring the universities to do more with less.

Student numbers are now some 18 per cent greater than a decade ago (at roughly 170,000). Within this total, there has been a widely welcomed increase of the proportion of women (up from a third to 45 per cent). Since the abolition of university fees in the 1970s the most dramatic growth has been in the numbers of part-time students in the university population: more than a third of undergraduates and 60 per cent of those studying for higher degrees are part-timers.

The universities differ among themselves both in size and in the general character of the courses they provide. The University of New South Wales (mostly in Sydney) is the largest, with 19,000 students, with the Universities of Sydney (18,000), Melbourne (16,000) and Queensland at Brisbane (18,000) not far behind. But even some quite new universities, such as Monash (founded in 1958, now with 14,000 students) and Macquarie (founded 1964, with 11,000 students), at Sydney and Melbourne respectively, have been able to grow rapidly. At the other end of the size scale are James Cook and Murdoch (in Western Australia), with 2,000 and 3,000 students respectively.

During the past five years of near-stagnation, the general response of the larger and thus more flexible universities has been predictable — to concentrate teaching and research on the stronger departments, closing down those that have a lesser contribution to make, and to seek external income by means of research contracts. The decisiveness with which the larger establishments have been able to respond to these pressures would delight the

British University Grants Committee, which for similar reasons in different circumstances is asking for exactly these responses.

In Australia, the Universities Council (one of the three statutory components of CTEC) has done what it can to strengthen good departments by persuading the universities to found ten "Special Research Centres", intended to be centres of excellence within the university system as a whole. (One of the ten centres, a nerve-muscle research centre at the University of New South Wales, was closed soon after being founded in 1982.)

The conditions under which these centres have been founded are exceedingly flexible; within reason, those appointed as directors are able to spend the funds available (up to \$A10 million over five years) on staff and equipment or some blend thereof. Academics and their critics appear to agree that an extension of this scheme would be valuable.

There is much less agreement about steps recently taken by the Universities Council to ensure the efficient administration or research funds in universities. The council's chairman, Professor D.N.F. Dunbar, wrote to vice-chancellors two months ago asking that universities should not "distribute by formula" this component of their recurrent budget, and including the ominous statement that "the present approach, which characteristically allows staff to follow their individual interests" is unlikely to make "the best use of the limited funds available".

For what it is worth, the successful universities are already seized of this need, while the successful university departments are fully aware that the success depends on the recruitment of outside funds. It is not for nothing that Professor Harry Messel, head of the School of Physics at the University of Sydney, boasts of having raised \$A30 million in as many years. □

Australian National University

A bed of "tall poppies"

AUSTRALIA's most unusual university, the Australian National University (ANU), is not so much a university in the ordinary sense as a collection of specialized institutes of advanced study to which there was added (in 1960) an undergraduate teaching establishment fashioned from the pre-existing University College of Canberra.

ANU is financed directly by the federal government, not through CTEC, as are the remaining universities, and for that reason is jealously regarded by academics elsewhere. The jealousy centres not merely on the separate flow of money (\$A135 million in recurrent costs) but on the terms of reference of those ANU academics in the seven research schools (Biological Sci-

ences, Chemistry, Earth Sciences, Pacific Studies, Physical Sciences and Social Sciences), who are required only to prosecute research, and who teach by grace and favour, sometimes at other universities.

Over the years, ANU has turned out to be a powerful means of attracting academics from overseas to settle in Australia. Inevitably, in an egalitarian land where "cutting down the tall poppies" has become the general idiom for the levelling process, ANU has become a focus for controversy. The excellence is not disputed, but academics elsewhere increasingly argue that they have to be excellent to succeed, and have to teach as well.

ANU's defence is twofold: that there is a small army of graduate students, drawn



from all over Australia, and that, in any case, many members of the research schools help out with lecture courses provided for the faculties. But only the research school of chemistry (at the instance of Professor Arthur Birch, the school's first director) is actually contiguous with its undergraduate department.

There is also a certain clubbiness about the relationship between the federal government and ANU. Last month, one member of the university described in a quite different context how he had been able to win support for a project by inviting a minister to a dinner. The research school of chemistry was apparently brought into being at a dinner in London (England) at which Sir Robert Menzies (then prime minister of Australia) and Professor Arthur Birch were both present. If those who would cut down tall poppies can be persuaded to stay their hands, there may be an interesting future.

The other easy complaint to make against ANU is that, not being part of the university system as a whole, it is not subject to the pressures of which other Australian universities are repeatedly reminded, that an academic's first responsibility is to make Australia prosperous. ANU's answer is quick and disarming: we do. And broadly speaking, ANU is right.

The research school of Earth sciences has Synroc to boast about; Australia has high hopes that this will become the world's preferred way of sorting radioactive waste. The School of Physical Sciences has spawned what must be one of Australia's most unusual companies, Optical Fibres (Research), which has received a grant of \$A5.4 million from the Industrial Development Board to develop a range of small sensitive sensors based on the fidelity with which plane-polarized radiation is transmitted uncorrupted through optical fibres subjected to various external conditions. Significantly, this development is an offshoot of the department of applied mathematics at the school.

To a hurried visitor, there is no difficulty in understanding why ANU is attractive to those who work there, or in accepting the claim that the institution has fulfilled the hopes of the university's founders that it would become a creative force in Australian intellectual life.

With the passage of time, and especially as the research schools at other universities have been strengthened, collaboration between ANU teams and others has become prominent, and should grow. To the extent, of course, that the need for such an institution has been diminished by developments at other universities, the question naturally arises whether ANU should continue indefinitely to exist. The simple answer is that it would be unthinkable, even in Australia, to cut down such a tall poppy; the best solution must be to turn the institution into a truly integrated university, eroding the present gulf between teachers and researchers. □

Research support

Catholic scheme for grants



AN academic scientist without a grant is like a magician without a silk hat (from which to produce rabbits); he may be able to manage but there is a danger he will not try. That is one reason for sharing the alarm now voiced annually by the Australian Research Grants Scheme (ARGS), the only source of funds for research in general, as distinct from biomedical research or one of still narrower sectors (marine science, for example) to which the Commonwealth government has accorded priority. Each year, the managers of the scheme (academics to a man, but working to the Ministry of Science) ask for more with a despair that outdoes preceding displays of outrage.

The scheme dates from the recognition 20 years ago that Australian universities had somehow equipped themselves, against earlier traditions, with people capable of research of the highest standard, and of teaching graduate students. In 1965, the federal government thought that £A1 million (then the Australian currency) might suffice, which it may have done. Now the complaint (familiar elsewhere) is that the budget has shrunk to less than half of what is needed to support all worthwhile projects. This year (1985) only a quarter of the new applications judged worthy of support won grants.

Everybody, even the government, seems to agree that this is unacceptable; but then nothing happens.

With appropriate adaptations to Australian geography, the scheme is much like those in operation elsewhere. People apply for grants at the beginning of each year, all applicants are interviewed (by an itinerant subcommittee) and grants are awarded on the recommendation of a committee (chairman, Professor Peter Sheehan, University of Queensland) by the Minister for Science. Applicants may be from any discipline, and need not even be academics (but invariably are).

The committee in charge does not mince words. "Funding for ARGS is dismal", it says in the introduction to the report on this year's allocations, after cheekily quoting Prime Minister Bob Hawke as having said a year ago that his government intended to support "excellence in research wherever possible".

Part of the trouble seems to be that the government's own rhetoric about the importance of research has increased grant applications while the budget has merely kept pace with inflation. This year, there was \$A23.9 million to spend on 2,359 applications that would have cost \$A66 million. As a consequence, according to the committee, 15 per cent of the 1,000 plus projects begun in earlier years had to be brought "prematurely" to an end. In constant money, the present budget is less than that a decade ago. The average

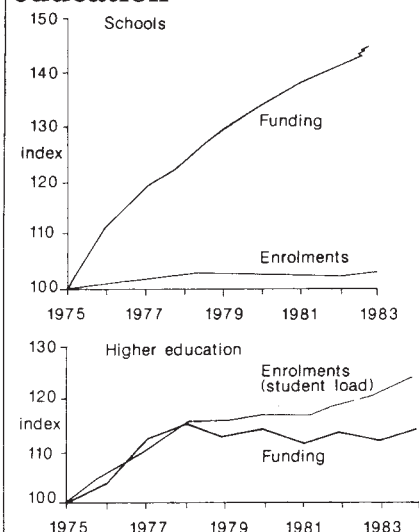
annual value of a grant has fallen to \$A19,478, compared with \$A24,475 (at 1984 prices) in 1975.

There is more to these complaints than empire building. The committee considers that its function is to cater for academic research in the round, whence the mixture of programme and project grants, equipment grants and career fellowships (for postdoctoral people). It rightly boasts of having backed a number of important projects, the programme for the *in vitro* fertilization of animals (including humans) among them.

For the years ahead, the committee had identified a number of new directions on which it would embark. Modestly, it asks for an extra £A3 million for equipment for 1986 (to double that spent this year) and money for supporting 30 postdoctoral fellows and 20 postgraduate students (to be attached to the better projects). It would like to be able to offer the ablest applicants a measure of long-term support and asks for \$A500,000 to spend in 1986 on schemes that will help to put Australia on the international intellectual map.

If everybody agrees that ARGS is a deserving cause, and that it is run well, why does its budget grow at such a modest rate? In the climate of Canberra, political explanations are unavoidable. Why should politicians increase the budget of an existing organization and run the risk of being called spendthrift when they can just as easily commit a few million dollars a year to some special scheme (as well as for marine science, there are now special grant-making schemes for biotechnology, energy research and water research) and gain the credit for a new initiative? Surprisingly, nobody complains at the earmarking of funds, but people say that the

Support for higher education



In recent years, the funding for undergraduate education has slipped behind that for schools.

multiplication of grant-making committees is a nuisance.

On or just above the horizon, however, there are at least three ways in which the future of ARGS will become a political issue. The most immediate is the demand by the chairman of the Commonwealth Tertiary Education Commission that ARGS should be administered alongside the universities (see p. 192). The argument is that it would be simpler that way. Academics seem to agree with Don Aitkin, professor of political science at the Australian National University and deputy chairman of ARGS, that the demand has been made only to be bargained away in return for some other concession at a later stage. They may have reckoned without Mr G.S. Hudson, the commission's chairman.

The ARGS budget, although less than 10 per cent of that of CSIRO, also touches the credibility of the science minister, Mr Barry Jones. The Hawke government is generally thought to have committed itself to increasing the budget by 10 per cent a year, and for 1984 did better. But last year, Mr Jones emerged from the budget fixing with only 6 per cent extra for ARGS. The outcome may say more about the relationship between Mr Jones and his colleagues than about the government's commitment to science (see page 188), but many see it as an election promise abandoned after victory at the polls.

Moreover, even academics who do not need ARGS or who are prevented from using it (as are those at the Australian National University) think the budget a disgrace. ASTEC has consistently said the funds available for research should be increased. No doubt its forthcoming report on research in Australia will repeat the message, perhaps more persuasively. People relate with black glee the tale of how in 1976 the grants budget was arbitrarily reduced because the then minister had not understood the difference between the financial and the grant-making year, which, they imply, just shows the indifference of governments to research. Unless the government is careful, ARGS could become an issue that divides it from the technical community.

There are alternatives to merely spending more. The Tertiary Education Commission's scheme for endowing centres of excellence (if continued and increased) is a means by which young able people can become established. Even the earmarked schemes can get people started, while it may seem to a government repeatedly made aware of over-academic university systems elsewhere that it may reasonably calculate the maximum restraint is the best policy, at least for the time being. The dilemma it must resolve is whether it makes sense to continue producing potential researchers whom industry will not employ and who cannot pursue their craft with government funds.

The scheme's policies are catholic.

While most successful applicants are at universities, the scheme can be used by people working at, say, state museums. Emeritus professors have a chance. The committees try to concentrate on certain fields (such as ionospheric physics or the mathematics of category theory, pioneered at the University of Sydney).

Grant applications reflect laboratory housekeeping needs. More than 60 per cent of the total sum awarded goes on salaries (for research assistants), less than \$A1 million (in 1985) is for applicants' travel, almost all of it within Australia.

Among the scheme's university clients,

successful researchers say that awards are generally too small, and do not obviate the need for supplementary funds from elsewhere. There is also a certain amount of grumbling by people in the natural sciences that the humanities and social sciences should consume nearly 20 per cent of the total, and on the other side of the fence, shock-horror at the sums spent on physical equipment. But the committees awarding grants insist that they work well together, and that ARGS should not be split. A better course would be to bring all the government's research grants schemes together. □

James Cook University

University of the Tropics



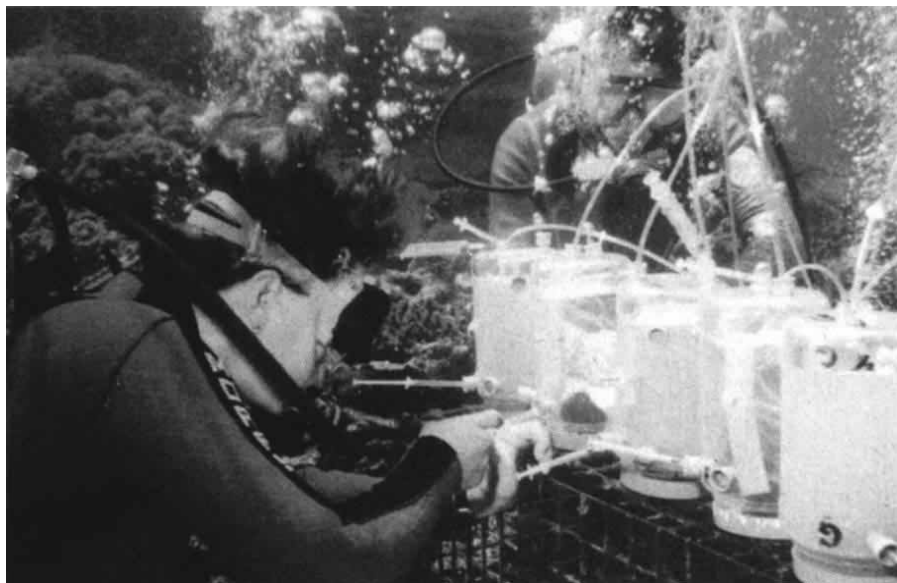
ONE of the occasional hazards of the James Cook University is to come in in the morning and find seven feet or so of python wrapped around a pipe in one's laboratory. The trick, apparently, is not to panic but to unwind it (or even better, have someone else unwind it) and put it somewhere useful, which according to Professor Campbell, of the Tropical Veterinary Science School, would best be the hay-shed, where it can perform the useful service of keeping down the mice.

Pythons, and some other snakes on which it would be singularly unwise to tread, well demonstrate what foreigners sometimes forget: that much of northern Australia lies firmly in the tropics (Australians simply forget that anybody lives there). Townsville, where James Cook University is located, is actually just 19° south of the Equator; close by are both arid tropical areas and tropical rain forests hardly different from those of Java. These environments, plus the Great Barrier Reef — 2,500 or so reefs stretching 2,000 miles along north Queensland's coast, with Townsville lying roughly at the centre — give James Cook University its special

character. It is the only university in the developed world that lies in the tropics, and has immediate access to tropical ecosystems.

The university itself is quite small, with just 3,000 students, but it has a unique facility in its tropical veterinary graduate school, set up in 1969. Against a background of declining university expenditure, the school has been able to expand steadily as demand for trained researchers in animal health and production has grown throughout the tropical world. At present, there are 60–70 graduate students with almost as many from South-East Asia as from Australia. There are close links with Indonesia and some 14 scientists work full-time at a research institute for animal diseases set up by the university at Bogor, with funds from the Australian Development Aid Programme.

Of major significance is their work on the filarial disease onchocerciasis, which in humans can cause blindness. Because filarial disease is also widespread in local cattle, in which it causes no more than lumps in the muscle, potential anti-filarial drugs are being tested in a pro-



The underwater laboratory developed at James Cook University — sponges are being incubated at a depth of 10 metres.

gramme supported by the World Health Organization.

Elsewhere, everyone seems to be making use of the opportunities offered by the Great Barrier Reef. The biologists, who offer the only degree course in marine biology in Australia, have progressed from completion of coral taxonomies to studies of reef community structure, reproductive ecology and population dynamics. Physicists have been modelling storm conditions, and engineers designing structures to keep out the box jellyfish (sea wasp) which makes swimming dangerous for more than half the year. Altogether, the university has been able to produce well over 100 papers in marine biology, from 11 different research sections, in a single year.

Even the chemists have managed to exploit the reef. Marine animals are an almost untapped reserve of natural chemical products, many of which have pharmaceutical potential. Early work was triggered by the Roche Institute of Marine Pharmacology at Sydney; with that institute's demise, interesting looking chemicals go to a US pharmaceutical company and the thrust of research has turned to finding out why the soft corals are so good at not being eaten by predators. (The answer is that they are full of terpenes,



A novel approach to university architecture at James Cook.

which not only deter predators but can be released to kill nearby hard coral, allowing the soft coral to overgrow it.)

The university even has its own miniature marine institute in the shape of the Sir George Fisher Centre for Tropical Marine Studies, named after the university's first chancellor. Here again, there is interest in natural product chemistry, but with a novel twist — the species studied cannot be kept in an aquarium so the laboratory bench is moved to the sea bed. The centre also keeps the Australian Collection of Marine Micro-organisms, as yet virtually unknown outside Australia, including more than 170 blue-green algae and two hundred bacteria, including petroleum degraders.

For geologists, the university's attraction is not so much the reef, but that half the nation's mineral resources lie north of a line drawn through James Cook, a region with no other university to serve it. To exploit their situation, the geologists have set up an Economic Geology Re-

search Unit (EGRU). In return for an annual membership fee, the hundreds of exploration geologists who roam the north can find a home to analyse samples and consult maps. Although EGRU is essentially one largish room equipped with geological and topographical maps and Landsat images, access is also given to departmental facilities including a scanning electron microscope. The centre has clearly been a success; numerous companies have signed up, which in turn has helped recruit funds for work carried out in the department, providing input into an MSc course in exploration and mining geology.

This neat tie-up with business does not,

University of New South Wales

Appealing to industry

SYDNEY'S "other" university, the University of New South Wales, complains like the rest of them at the steady erosion of its budget (by an estimated 2 per cent a year). But it is big enough, with 18,000 students, to be flexible in the management of its affairs. It also has the unique benefit of an agreement with the federal government to operate the Australian Defence Force Academy, more than 100 miles off-campus at Canberra.

Chartered only in 1952 as a technological university, the institution has a flavour of diversity. As well as medicine there are faculties of applied science, architecture and law. Humanities are big, while the arts faculty is both a leavening for the rest of the curriculum and a valuable contribu-

tion to Sydney's cultural life. However, prevent the head of the geology department, Professor Bob Carter, from pointing out Townsville's big disadvantage — isolation that means political isolation from the decision-making and grant-giving heart of Australia and, more important, isolation from fellow scientists. With 12 full-time staff, 15 PhD students plus a few research fellows and a yearly travel budget of just \$A2,500 (a return ticket to Sydney costs \$A700), the complaint is natural. Fortunately, the reef provides a bit of help. Now that Townsville has an international airport, quite a few scientists on round-the-world trips stop off to see the reef, dropping in at the university on the way. **Alun Anderson**



tion to Sydney's cultural life.

Financially, the university continues to grow, but only by means of research grants and donations. Although the federal government's grant (at \$A125.6 million) was unchanged between 1983 and 1984, the administrators complain that the inflation factors used by the Commonwealth Tertiary Education Commission do not adequately compensate for the inflation of academic costs. But the university is now raising more than 21 per cent of its income from other sources, most of them public funds such as the ARGS (see p.194). There is, however, an invaluable \$A4 million income deriving from investments, a pot of gold worth \$A54 million (which should surely be yielding more).

The university typifies those among the larger universities who see their future as one in which they will be increasingly dependent on what they can earn by research and other means. There is, for example, a university company (called Unisearch Ltd) which promotes the role of its academics as industrial consultants, and which also handles joint research contracts and even the exploitation of patents; gross income last year exceeded \$A2.58 million.

Not surprisingly, the search for outside funds runs into the familiar conflict between academic and commercial interests. Professor Raymund Golding, the pro-vice-chancellor for administration, says in the annual report just published that potential industrial partners cavil at the payment of an element of overhead costs and also think they should in some sense "own" the results of successful projects. If what people say about Australian industry's regard for research is true, the learning process will be slow.

The search for outside sources of funds is one of the reasons prompting the creation in the past few years of a series of topic-based interdisciplinary centres with responsibility for teaching and research. Uniquely, it is claimed, there is a centre for law and technology, and others dealing

Popular science

MAKING science public in Australia falls to a variety of agencies, of which the best known and most frequently disappointing is the Australia and New Zealand Association for the Advancement of Science (ANZAAS), whose 1985 meeting will be held next month in Sydney. The Australian Academy of Science does a valuable service, but of a different kind, having become over the past decade a first-rate school textbook publisher.

But Australia's most vivid source of news about science is the Australian Broadcasting Corporation's output of sound radio, and in particular the series of regular programmes built around the weekly programme *Science Show*. The producer, Robyn Williams, says that ABC has given him the chance during the past decade to try a variety of formats. The programmes are both popular and arresting. Especially now that they are complemented by programmes ranging from straight-forward but informal talks to general information about the use of computers, they have contributed to the high awareness of policy on science and technology in Australia. □

Kangaroos thriving but still threatened by drought

DESPITE rumours to the contrary, the kangaroo is alive and well and living in Australia. In recent years, there has been considerable concern, and international pressure, over their commercial killing and some fears that kangaroos could be endangered. Although criticism over hunting methods may still stand, sophisticated aerial surveys show that there are still plenty of kangaroos.

"Kangaroo" is not in itself a scientific term and generally refers to the man-sized members of the Macropods, usually the red kangaroo (the favourite at zoos) and the two grey kangaroos. Among smaller members of the group, the wallabies and rat-kangaroos, there are six endangered species and several others that are rare, although none is commercially hunted.

It is almost two hundred years since the kangaroo was first exhibited in London, at the Lyceum in the Strand; admittance was seemingly expensive at one shilling, but perhaps not for "an Ocular Demonstration [which] will exceed all that words can describe, or pencil delineate ...". And from that time, kangaroos have been killed for meat, for their skins or simply as pests, by gun by poison or by simply rounding them up and clubbing them to death by the hundreds, in the "battue" that occupied sporting Australians in the nineteenth century.

More recently, kangaroos have become a subject of fierce controversy; some would have them totally protected as a unique symbol of Australia, some believe they should be destroyed as pests because they

damage fencing and sheep pastures, while some hold that they should be managed for maximum profit as a renewable resource. International pressure has come from the United States where conservation groups succeeded, in 1974, in having red and grey kangaroos listed as a "threatened species", and the import of kangaroo products banned. The ban was later lifted after Australia was able to demonstrate that kangaroo



resources are properly surveyed and managed. All seemed set for them to be removed from the US list of threatened species but in April 1984, the US Fish and Wildlife service ruled that the severe 1982-83 Australian drought had put the status of kangaroo populations in doubt once more.

The latest evidence suggests, however, that kangaroos may be even more abundant than before European settlers ar-

rived. Thanks to work by Graeme Caughley of the CSIRO Division of Wildlife and Rangelands Research, kangaroo populations can now be accurately estimated from the air. The trick is to fly a series of transects, at a particular height and speed, counting the kangaroos within a 200-m strip marked by streamers trailing from the aeroplane. Not all kangaroos are seen but a set of equations, devised by Caughley is used to correct the observer error according to the type of countryside. When these results are compared with sightings of kangaroos recorded in early explorers' diaries, it does seem that the kangaroos are more abundant than ever before. The probable explanation is that the provision of water-holes for sheep and the reduction of the predatory dingo population have removed constraints on the population.

Commercial taking of kangaroos is now very strictly controlled in all states. Unlicensed hunting is prohibited. But it is unlikely that protest will diminish over the seemingly brutal way in which kangaroos are taken by dazzling them at night with bright lights and finishing them off with clubs. Such practices have gained international notoriety; when the Japanese were under fire last year for whaling, from Australian conservation groups among others, Japanese newspapers ran pictures of Australian kangaroo hunting with a headline raising this awkward question "How can they criticize us?" This is a point the Australians might take.

Alun Anderson

with the ophthalmology of contact lenses (a local speciality), industrial relations and the Japanese economy. To label a group of academics thus may either be a way of simplifying the task of giving outsiders special advice in the form of custom-designed courses or a way of attracting research contracts credibly.

One of the most conspicuous of these centres is also the most unusual, consisting of a collaboration between the university and the Royal Melbourne Institute of Technology. (Sydney and Melbourne are generally linked together only by their rivalry.) The funds for the venture (\$A2 million) were originally provided (in 1982) by the federal government, then founding ten industrially oriented research centres at universities. More recently, the centre has made a deal with the Australian subsidiary of IBM which may not be a direct source of income but which extends the scope of the operations.

The centre's proudest boast is to have produced what are the most efficient solar cells so far to have been made of amorphous silicon (see M. H. Green *et al. Appl. Phys. Lett.* 44, 1163; 1984). The trick is to coat the surface of a piece of silicon (doped with phosphorus by diffusion from the upper surface) with a layer of silicon oxide between 20 and 50 Å-

stroms thick, using a technique developed by M. H. Green over the past decade. The insulating layer inhibits recombination of electrons and the holes from which they have been quarried; electrical contact through the upper, exposed, surface of the cell must be accomplished by a network of metallic fingers evaporated onto the surface. The highest efficiency claimed so far is 19.1 per cent (with an open-circuit current of 0.64 milli-ampere).

Nobody pretends that hand-built cells like this (the standard size is 2 cm square) will be mass-produced so as to meet the world's (and Australia's) need for energy. The next goal, at the centre, is to push up the efficiency to 20 per cent or more. In the process, the centre argues, people will learn to understand what design parameters are needed to build an efficient, and cheap, solar cell. And in any case the laboratory will become even more familiar than at present with the technology of making semiconductor devices; it has ambitions to use thin films of insulators in more conventional transistors.

The facilities at the centre are not very different from those to be found nowadays in microelectronics laboratories elsewhere in the world; the University of Edinburgh in Scotland is, if anything, better equipped. But the joint centre gives

the University of New South Wales (and its Melbourne partner) the chance to acquire and improve basic skills in an important field and, with luck, even the chance to strike gold one day. The federal government's investment seems to have been worthwhile; next month's budget will show on what scale it is to continue.

For the university as a whole, this is only one of a great many developments that should collectively give the lie to the federal government's suspicion that Australian academic institutions contribute too little to social and economic well-being. Visitors from a land of small universities such as Britain are bound to be impressed both with the financial robustness and the intellectual diversity of a really large university. New South Wales is large enough to be flexible about student entry (more than a quarter of the students are part-timers, a proportion growing towards a third are women) and even to mount special programmes for aborigines and students from other sectors of higher education without formal academic qualifications. It is also large enough not to be ashamed to have an international reputation both in the anatomical description of the mammalian retina (as by Dr Jonathan Stone's group) and the mechanical and optical engineering of contact lenses. □

Adelaide University

The sweet smell of success

THE University of Adelaide's compact campus embodies the whole range of responses to the overall decline of funds for basic research facing Australia's scientific community. Barring a last-minute reversal, the university is switching resources away from science; seven posts are to be taken from the Faculty of Science this year, and transferred into commerce, law, women's studies and engineering. But, at the same time, the university has set up its own venture development enterprise and encouraged new joint ventures with industry, which are now flowering in a number of departments.

Those whose subjects are currently in vogue find little to complain of, and several departments have managed to win various species of the government's new grants. But for those in areas of basic research now out of fashion, or without immediate appeal to industry, the outlook is bleak.

Physics is likely to be one department affected by the shift of jobs to elsewhere in the university, even though the demand for physics graduates is as high as ever, according to annual surveys conducted by the department's head, Professor John Prescott, and despite a recent series of major discoveries by the cosmic ray group. Using the only detector of energetic cosmic-ray showers in the Southern Hemisphere (home-built at a fraction of the cost of others), they have discovered two of the three known sources of ultra-high energy X-rays — binary X-ray stars in Vela and the Large Magellanic Cloud (see *Nature* 315, 205; 1985).

Good work is also being done by the atmospheric physics group which has installed the first middle-atmosphere radar in the Southern Hemisphere, and by a group interested in the solid-state physics of thermoluminescence dating. They now claim the most sensitive thermoluminescence spectrometer in the world. Not surprisingly, there is a deep sense of frustration that sufficient funds are not available to build on the department's achievements, nor to stop talented workers slipping away to the United States.

Professor Prescott sees Australia's level of spending on science as "appalling" and points out that Australia is seventeenth among the 21 OECD countries in terms of research and development spending as a percentage of gross national product. But that does not mean that all hope has been abandoned: one member of the department, fresh from CERN, is campaigning against all odds for Australia to take up high-energy physics.

A more optimistic outlook on life prevails in the geology department, where Professor David Boyd has just had the go-ahead (and \$A150,000 a year) for a "key" centre, one of the Commonwealth

Tertiary Education Committee's new concepts to promote areas of "special economic and social significance". The key centre is for advanced studies in petroleum geology and geophysics; ten people a year will enter a two-year course and there should be plenty of jobs waiting for them, given the probable acceleration in the pace of oil exploration. Professor Boyd has spent a major part of his own career in industry and the new course will involve the Australian Mineral Foundation, an industry-financed independent institute running advanced workshops. The aim is to mix students with other people in industry; that will, in turn, help industrial funds flow into the department.

In biochemistry, there is also the sweet smell of success. A "centre of excellence" was created in 1982, with a \$A1.2 million grant over three years; this has now been followed by \$A1.6 million for the next three years. The Commonwealth Special Research Centre for Gene Technology, as it is officially known, carries out fundamental research on gene regulation under Julian Wells.

Within that huge area, there are projects on the activation of genes that metabolize drugs in the liver, the isolation of wool keratin genes, plant viruses and viroids, tissue-specific expression of histone genes and the use of recombinant DNA technology to produce growth hormone. Virtually all the projects are planned with some direct application in mind. The drug metabolism studies relate to the human disease porphyria. Studies on wool show cysteine to be a limiting factor for growth, so the ideas of introducing cysteine synthesis genes by embryo microinjection is being explored. Viroid probes can allow



the early detection of economically important plant diseases, and so on.

Indeed, the group has already gone into the commercial business by setting up BRESA — a wholly university-owned biotechnology company. Eight full-time researchers are already at work for the company and have one best-selling product — photobiotin — which allows the simple light-activated coupling of biotin to DNA and RNA probes.

The microbiology department also hit the jackpot last year with the acquisition of a \$A870,000 grant over three years from the government's new National Biotechnology Programme. The grant went to Professor Derrick Rowley for a collaborative project with industry — in the shape of F.H. Faulding and Co. Ltd, a successful local pharmaceutical company, for the development of oral vaccines against enteric diseases. A joint company "Enterovax" has been set up, employing 14 people on research within the microbiology department.

It is not strange that the biologists are now getting the cream of the grants, for the government has to some extent recognized the potential of biotechnology. (Biologists also tend to mutter that physicists had their day of riches in the past; now it is the biologists' turn.) But that said, only seven per cent of applicants for funds from the National Biotechnology Programme received anything at all, an astonishingly high rejection rate which has led to complaints about the programme content, the level of financial support and even the competence of the grant authority. Competition for "key" centre funds was also severe with some very disappointed applicants with excellent schemes. Adelaide University (or at least some parts of it) must therefore count itself among the blessed.

Alun Anderson

Wildlife research

Vermin are out — fauna in

AUSTRALIA'S interior contains a veritable menagerie of the descendants of settlers' domestic animals. Besides the rabbits, there are wild donkeys, horses, goats and pigs; enough camels to provide for a roaring trade in their export to Saudi Arabia; and, in the tropical wetlands east of Darwin, around a half million water buffalo. It is not surprising then, that the principal wildlife research organization, CSIRO's Division of Wildlife and Rangelands Research, carries out an inextricable mix of research on pests and on wildlife behaviour, ecology physiology and conservation.

The division is itself a recent marriage; the Wildlife and Rangeland Divisions were formerly separate, but it was felt that in these days of ecosystem studies, there was no further virtue in keeping plants and animals apart. The division provides for

long-term fundamental studies; shorter-term and day-to-day management problems of conservation areas remain in the hands of the park and wildlife services of the states.

Australia is well covered with parks of one kind or another. Of the total land area, 3.7 per cent (28 million hectares) is specifically reserved for nature conservation. State by state, the proportion of land set aside for conservation varies greatly, reaching 25 per cent in the tiny Australian Capital Territory and 14 per cent in Tasmania, but is less than 1 per cent in the sparsely populated Northern Territory. The Great Barrier Reef is protected by a specially constituted Marine Park Authority.

The division responds to requests for fundamental research from the various states, and also takes on work funded by





Laying down the law on wildlife protection

TRAVELLERS beware! On your stop-over in South-East Asia on the way to Australia, do not be tempted by souvenirs of ivory, exotic butterflies, bird feathers or shells. If you succumb, a gaol sentence may await you.

From May 1984, Australia has had what are probably the world's strictest laws regulating the export and import of wildlife and plants. The native fauna of the nearby developing countries could be ruined by tourists prepared to pay fabulous sums for exotic souvenirs. But the new act provides for up to five years' imprisonment and a \$A200,000 fine for those so tempted.

Indeed, the new laws were considered so strict that \$A0.25 million was spent publicising them before they came into effect — and two gold medals for the best advertising campaign of the year were won in the process. Australia's action will undoubtedly help stop regional trade in endangered species; if Japan could be induced to take similar tough action, real progress might be made.

Responsibility for administering the Wildlife Protection (Regulation of Exports and Imports) Act lies with the Australian National Parks and Wildlife Service

(ANPWS), set up in 1975 as the principal adviser to the Commonwealth government on national nature conservation and wildlife policy. This duty includes the job of representing the government at CONCOM, the council of nature conservation ministers drawn from all the states, and on international bodies such as the International Whaling Commission.

ANPWS also has responsibility for some 5,000 galleries of paintings — all of them out of doors, on rock, and some 40,000 years old. That makes them by far the oldest paintings in the world, and according to Professor J.D. Ovington, the service's director, they are also "among the most beautiful".

The paintings are all in Kakadu National Park in the Northern Territories which, along with Uluru Park (Ayers Rock and Mount Olga) and Christmas, Norfolk and other island territories, are administered directly by ANPWS.

The aim is to set standards that state authorities will emulate (although some may not like to hear that). Certainly, considerable praise has been won for the Kakadu Park's involvement with the local aboriginal community; older people have

been appointed as cultural advisers and many younger people have been trained as park rangers.

ANPWS also has sufficient funds to assist directly conservation programmes of national significance. Needs are diverse and last year the service helped more than thirty projects, ranging from a national plan for dealing with mass strandings of whales to the use of airborne scanners to map vegetation. Odd situations arise; when Vietnamese refugees with quite different eating habits arrived in the country in large numbers, there emerged a risk that they might decimate some marine animals that Australians had not thought of as food.

On a more mundane level, ANPWS produces a staggering output of educational literature — part of which includes the postcards shown above. Indeed, if the number of posters and brochures on Australia's unique wildlife and the masses of endangered species leaflets and stickers are anything to go by, exemplified by the postcards shown above, Australia is well on the way to becoming the world's most conservation-conscious nation.

Alun Anderson

the various agricultural product boards and the like. Some 12 per cent of its \$A8 million budget comes from such outside sources.

Thus the Wheat Board is now supporting research on one of Australia's more bizarre problems: the so-called "mouse plagues" that erupt at four to twenty year intervals in the wheat belt of south-eastern and eastern Australia. Between plagues, mice are rare and of little economic account. When a plague gets under way, they appear locally in their millions producing, as one surrounded householder put it, "a moving carpet of mice, in some places three and four deep... when you walk you tread on them and they run up your legs".

The mice eat everything they can; crops, seeds and the wiring and upholstery out of cars, tractors and harvesters. They penetrate through wooden walls, devour furniture and clog up irrigation pumps. In a 1979 plague in Victoria, \$A15–20 million

was counted, but that is probably an underestimate; a considerably less severe plague in 1984 caused \$A30 million worth of damage.

The problem is perplexing, for the plagues are local and sporadic. Work by Trevor Redhead at the division indicates that the essential trigger for a plague is above average rainfall in autumn which lengthens the time for which the mice can reproduce in winter. But the mechanisms by which the population build-up can reach "plague" levels is far from being understood.

The division's work is not all plagues and pests, however. Basic work by L. Braithwaite on tree-dwelling marsupials, the possums and gliders, suggests that changes in logging practice could reduce the impact of commercial forestry on their populations. The eucalypt forests in which the animals live are commercially exploited, and conventional wisdom has it that harm to wildlife can be avoided by

cutting alternate plots of land in a long-term cycle. But the real situation is not so simple: the marsupials prove to have very marked preferences for certain trees, preferring the so-called "peppermint" eucalypts. This turned out to be because the leaves of the peppermint eucalypt contain high levels of nutrients and so can provide more food for the leaf-eating marsupials and more insects for the insect-eating marsupials.

The Forest Commission has taken note of the patchy distribution of fauna, but a problem remains. The nutrient-rich trees grow preferentially on nutrient-rich soil, much of which, because of its economic potential as farmland, is in private hands. So as is common in wildlife research, the future of the possum and glider marsupials must be passed from scientists to politicians. A way to resolve a potential clash of interests between the needs for faunal conservation and agricultural production must be found.

Alun Anderson

Nuclear energy

The slumbering giant



WHATEVER can have happened to nuclear energy in Australia? A quarter of a century ago, the Australian Atomic Energy Commission, like all its counterparts, was busily designing a nuclear reactor, urging on the exploration for uranium (for which it was the government's sole agent) and even dropping heavy hints that peaceful nuclear explosions might be used to create harbours in the otherwise inhospitable west coast. Australia still contains the world's largest reserve of uranium ore, more than a quarter of the world's total, but otherwise there is a deathly silence.

What has changed? The political temper of Australia, which is not the same as party politics. Australia has acquired in the past decade a detachment from the world of powerful alliances. The close alliance with Britain, now seen as subordinate membership of the old Empire, has long since faded (and may entirely disappear when Judge McLelland's report on British nuclear testing in Australia appears later in the year). The brief flirtation with the United States in the 1960s (Australia was the only other country to send a force to Vietnam, and its then prime minister invented the phrase "All the way with LBJ!") has also withered, as Australia looks more closely around the Pacific rim.

Just over a decade ago, people in Australian television talk-shows were forever arguing about the wisdom of exporting raw materials to economic rivals; why not add the value here, in Australia? Better leave the minerals in the ground, some said. Since then, the long-standing Australian concern that others should not make weapons from its uranium has been strengthened, not least by the French nuclear tests in the Pacific. All political parties include a powerful section of opinion that uranium should be left in the ground. Mr Hawke's Labor Party is riven on uranium policy.

The dilemma for the present government is that the political risk of offending the opponents of uranium mining are offset by the political risks there would be in losing all those jobs, not to mention an export trade worth upwards of \$A2,500

million a year. (Coal exports, now running at \$A4,000 million, are worth even more.) That, no doubt, is why, in November 1983, Mr Hawke asked the Australian Science and Technology Council (ASTEC), and its chairman personally, to study "Australia's role in the nuclear fuel cycle".

ASTEC's response, published last May, failed to resolve the dilemma. The committee endorsed the traditional Australian view that uranium suppliers should do everything they can, by imposing conditions on the uses to which their uranium may be put, to inhibit the proliferation of nuclear weapons. But the council's report went on to say that exports should not be limited "as a matter of principle" but instead should be permitted under stringent conditions. And after endorsing government policy on the control of nuclear weapons (suggesting, in passing, that Australian bilateral agreements should be published as models for other suppliers) went on to argue for Australian participation in "stages of the fuel cycle other than mining and milling" if that will help strengthen the "non-proliferation regime".

Last summer's Labor Party caucus on uranium policy was apparently contentious. This may have been when Mr Barry Jones, Minister for Science, blotted his copybook by opposing the ASTEC recommendations (see p. 188). In the event, Mr Hawke was forced to announce the rejection of the two contentious proposals: mining would be allowed at only a third mine in South Australia if that proved economic, Australia would not participate in other stages of the fuel cycle and this decision will not be reconsidered at least until the end of 1987 (by which time there will have had to be another general election).

Opinions remain divided. The case for doing something to the uranium, enriching it in uranium-235, for example, had seemed strong enough until last year for the Atomic Energy Commission to be hard at work on the centrifuge enrichment process; now that programme has been converted into what is described by Dr D. G. Walker, the director of the commission's laboratory at Lucas Heights, north

of Sydney, as a study of the applications of anti-proliferation measures to the centrifuge process.

Against the background of the Hawke announcement, it is a little strange to find that the Lucas Heights establishment has shrunk since its peak a decade ago. There are around 1,000 employees; most of the fall from 1,300 a decade ago is explained by the transfer to CSIRO of the previous programme of research on unconventional energy sources. Yet nobody seems worried about his job.

Part of the explanation is Synroc, the name for the project that has grown from the advocacy of Dr A.E. Ringwood, from the Australian National University, that would incorporate highly-active waste in synthetic rock. Ringwood's process, not yet proven with radioactive materials of the kind for which its use is planned, has given Lucas Heights a new lease of life. The commission has signed agreements on collaboration with the United Kingdom and Japan.

For the rest, the establishment has three functions. It is a major source of short-lived radioactive isotopes not merely for Australasia but occasionally for more distant customers. (West German non-destructive testers used to buy substantial amounts of ytterbium-169.) More recently, the establishment has developed a neat way of extracting technetium-99 from the fission product molybdenum-99 in such a way that physicians can extract small amounts of this useful tracer when needed simply by eluting it from alumina.

Lucas Heights is also a general-purpose research establishment (in agriculture, hydrology and nuclear physics) and a teaching resource (with a 3-MeV Van de Graaff machine used chiefly for fission studies). What would be said of a uranium-exporting country that had no means of telling what fission is?

The third function is advisory. Simply because of the government's interest in proliferation, a small army of experts in the field is a necessity. This need is unlikely to vanish. And in any case, Lucas Heights is a nuclear development establishment in waiting, the kind of place that would be needed if a change of policy came along.

The critics of Mr Hawke's uranium policy are of two kinds, those who would leave the material in the ground and those who hold that it is too restrictive. The next dispute is likely to blow up when the commission asks for a replacement for its HILAR reactor, modelled on a British reactor built in the 1950s. Dr M.H. Brennan, the Sydney physicist who is also the commission's chairman, thinks the request cannot be long delayed. Others go much further, pointing out that there are already many remote parts of Australia where a cheap and small-scale source of nuclear electricity would be a boon. And the present economic upheaval may make many people regret the loss of all that value added. □

The leading producers of uranium (non-communist countries)

Country	No. of plants	Capacity (tonnes per yr)	Estimated reserves (tonnes)
United States	33	13,750	131,300
Canada	5	8,540	176,000
S. Africa	15	5,780	191,000
Niger	2	4,450	144,000
Namibia	1	4,250	119,000
Australia	2	3,950	474,000
France	6	3,330	56,200
Gabon	1	1,500	18,700

Figures for uranium production are as at June 1983, from estimates made by the Australian Atomic Energy Commission. Reserves are "reasonably assured resources" for 1983 based on a cost range to US\$80 per kg. Values for other countries other than Australia from OECD. No estimates are available for the Soviet Union, Eastern Europe and China.

Defence research

Marshalling scant resources



THE largest research and development complex in the Southern Hemisphere sits some 24km outside Adelaide. It has a staff of 2,660, five square kilometres of grounds, 1,300 buildings and many, many kilometres of high wire fence. It is the main research centre for the Defence Science and Technology Organization (DSTO) and appropriately enough, for those familiar with British military manoeuvres, is just beyond a town called Salisbury Plain.

Besides the Salisbury centre, there are some eight other defence research laboratories around the country, with a total staff of about 4,400, but with fewer research scientists than CSIRO. Annual expenditure runs at \$A140 million or 2.7 per cent of the defence outlay, which is itself 2.7 per cent of gross national product. That is just one-twentieth of the British defence research and development budget, a statistic which explains much of DSTO's character; it seeks to cover the whole range of technical problems even with a very much smaller capability.

Inevitably, most defence equipment is bought abroad rather than developed in Australia and a great deal of DSTO's energy goes into maintaining the ability to give advice on equipment needs as well as adapting foreign equipment to the very different operating conditions of the Southern Hemisphere. Indeed, the spread of knowledge required is such that, occasionally, all the expertise in one area resides in just one individual — to whom it is earnestly hoped that nothing untoward happens.

A little research capacity is, however, left over for the development of quite new concepts. Past successes include Malkara, the world's first wire-guided antitank missile, bought by the British during the Suez War of 1957 when they suddenly realized that they had sold the then impregnable Centurion tanks to the other side. And, after that, there was Ikara, a missile-borne homing torpedo still widely used.

Present public fantasies about DSTO's activities include, if the popular press is a guide, a "death ray" or, more precisely, an electromagnetic rail gun which the United States is said to be keen to see as part of its "star wars" (Strategic Defense Initiative, SDI) inventory. Rail guns fire metal slugs at very high speeds by electromagnetic pulses: one Australian scientist has done some neat work (in the United States) on the power pulses that provide that drive. But there is no major development programme under way and the possibility of involvement with the SDI programme is still a subject of fierce political debate.

There are, however, two big defence projects of worldwide significance. In one, Australia is up with the leaders. The Jindalee over-the-horizon radar system

based at Alice Springs can illuminate an over-the-horizon target by bouncing a high-frequency radar signal off a refracting layer in the ionosphere; the signal will return by the same route, and is picked up by a 2.6-km-long linear array. Clever signal processing is then needed to pick up the target against noise returned from hundreds of square kilometres of background. Essentially, flying objects are detected by their Doppler-shifted signals; ships can be picked up because they are stationary against a background scatter Doppler-shifted by moving waves.

The second project, Winnin, was aimed at building a decoy for Exocet-type anti-ship missiles long before their lethal efficiency was demonstrated in the recent Falklands War. The decoy is now at the "proven concept" stage, awaiting a foreign partner for further development, and was exhibited at the Paris Air Show in May. It consists of a remarkable rocket which can hover, nose up, at any desired height above the ground and which, by a slight tilt, can move from side to side, horizontally. The advantage of using a rocket as a decoy is that it can be launched in just seconds — after an Exocet has been detected, a maximum of two or three minutes are left before impact. The rocket can then hover and move very slowly sideways, just as though it were a ship, so that

the Exocet radar cannot readily tell it is a decoy. To complete the deception, the decoy would carry a sophisticated piece of electronics to amplify and return the missile's radar signal to lure it away from the smaller signal given by the intended target, the ship.

A host of other devices have been developed by the various branches of DSTO. These include the Karinga cluster bomb, the BARRA sonar buoy, and the LADS laser airborne depth sounder which can map 50 km² of ocean an hour with a scanning green laser beam that penetrates to the sea floor. Not everything is designed for purely military use: there is also an airborne neonatal intensive care unit for "flying doctors" and a cheap and tiny parachute which allows water bottles to be dropped from the air without bursting.

Financial pressures on DSTO seem not to have been as great as those affecting CSIRO and the universities. Australia's lack of a large-scale defence industry virtually assures that most research and development must remain within a large government organization, although close links are maintained with such defence industries as exist. Support is given to keep engineering expertise in industry from dispersing between contracts. For the future, the chief problem will be whether the thin skin of experts can be stretched to cover the ever-expanding technology of defence.

Alun Anderson

Australian Institute of Marine Science

Aiming to count starfish

THE Australian Institute of Marine Science (AIMS) at Townsville must be about the only research laboratory in Australia to be reaping some benefit from increasing unemployment. Thanks to the Commonwealth Community Employment Programme, \$A1 million has been found to give 55 people work on a one-year study of the distribution of the Crown-of-Thorns starfish on the Great Barrier Reef.

Other than this welcome inflow of money from a department not normally associated with science funding, AIMS faces much the same problems as other institutes; its budget, currently \$A7.3 million a year, is not rising, but inflation is driving overheads ever upwards. So far this seems not to have dampened the enthusiasm of AIMS's staff who, to the institute's great good fortune, are largely in their thirties and perhaps at the most creative part of their careers.

The institute too is young; it was set up 11 years ago under an act of parliament which gave it responsibility for research in the entire field of marine science and has been handsomely provided with facilities, including three research vessels, the largest of them 24.4 metres long. Uni-

versity researchers speak enviously of "luxurious blue-carpeted halls" (which really do exist), but as the institute is located 50 km out of Townsville, at the end of a 20 km private road, used mainly by hundreds of wallabies, the opportunities for sack and pillage are slight.

Despite the very wide brief AIMS was given, it is not actually able to send scientists very far afield. Research remains firmly linked to the Great Barrier Reef and to the tropical coastline of mangrove forests further to the north.

Current worries are that the reef may once again suffer a massive increase in numbers of the Crown-of-Thorns starfish, similar to that in the late 1960s and early 1970s. The starfish eat the hard-bodied corals, and the result of an outbreak, when starfish can be seen on a reef by their hundreds of thousands, is a change from 45 per cent coral cover to just two or three per cent, with accompanying catastrophic changes in the rest of the reef community.

That the starfish are once again on the increase is suggested by reef surveys and by questionnaires returned to the Great Barrier Reef Marine Park Authority by vacationing skin divers. For AIMS, however, what is most certain about the

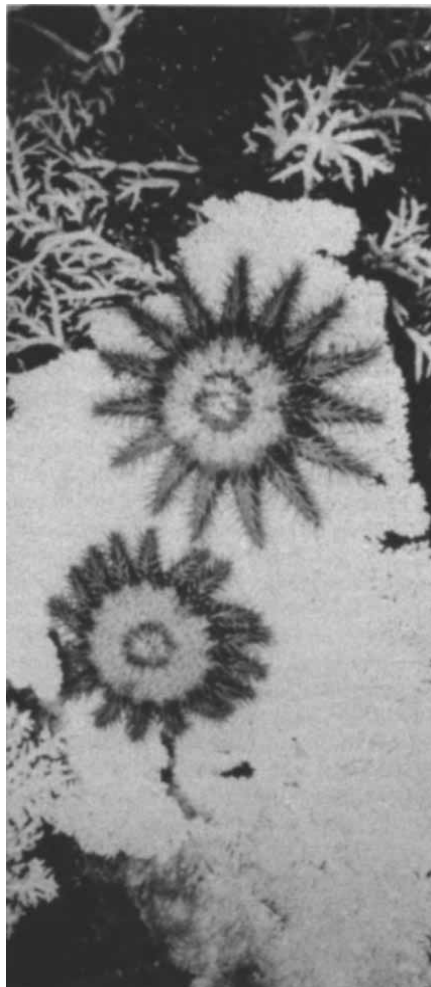
outbreak is the vast uncertainty. Nobody knows the true extent, or the dynamics, of the changes in the starfish population, hence the Department of Employment programme, funded to provide a one-year "snapshot" of the coral-starfish system. Two hundred and twenty eight types scattered along the whole length of the reef are to be sampled.

So far at least it does not seem as if the reef is heading for destruction. Areas known to have been decimated 10–15 years ago appear to have recovered their coral cover, although it is not known if the reef is quite the same as it was before. It is entirely possible that outbreaks of the starfish are "natural" and have been continuing for vast spans of time. Cyclic changes in starfish abundance with complex dynamics can be driven simply by predator/prey interactions, as recent mathematical modelling at AIMS shows. On the other hand, cycles may have been triggered by some external factor. Either way, the possibilities for control seem remote. In Japan, an attempt was made to prevent a Crown-of-Thorns outbreak by removing an astonishing ten million starfish from reefs in the Okinawa region, but without success.

Research on the Crown-of-Thorns starfish is but one (temporarily well funded) part of AIM's activities. Elsewhere, diversity is the name of the game, and AIM's activities well illustrate how basic research can confound the ill-informed among politicians and have consequences of economic and perhaps profound social value.

Why, for example, are coral reefs free from bryozoans, tunicates and so on that are found encrusting nearby piers and pilings? The answer proves very simple. In the clear waters of the reef, the levels of ultraviolet light are simply too high to permit their growth — put a sheet of ultraviolet absorbing Perspex on the surface and they will grow in abundance. (Those who hold the popular misconception that ultraviolet light cannot penetrate far in water should read N.G. Jerlov's 1950 *Nature* paper which shows quite the contrary.) The question then becomes one of why corals are not similarly affected by ultraviolet radiation. The answer proves to be that they contain a natural "sunscreen", a UVB blocking agent. This has now been isolated and seems set to form the basis of a commercial sunscreen, or even a marine plastics and paints additive. Results of the research are being held back from publication until formal patents have been filed; a contract with a chemical company to develop the agent has already been signed. Preliminary announcements were feted by Barry Jones, Minister for Science.

Results with broad economic consequences could also stem from a second study (published in part by *Nature* last August) which began when it was found, contrary to received wisdom, that fluorescent



The Crown-of-Thorns starfish again threatens the Great Barrier Reef.

bands appear in sections cut from long-lived *Porites* corals. The bands, in corals growing on the shore side of the reef, turn out to be caused by fulvic acid leached from soil by water; they thus provide a record of nearby river output, and so of rainfall, which can be dated to within a few weeks by X-ray examination of the annual growth rings of the coral. As massive corals may be many centuries old, they thus contain a long-term record of floods, droughts and river flow — exactly the sort of information that saves huge sums of money in the planning of dams and flood control systems.

As well as showing annual cycles, the bandings record much longer-term patterns of atmospheric pressure. The record in the corals has the potential to push knowledge of Australian meteorological conditions back several centuries. What makes this so important is that the region is critical for the Southern Oscillation, the unpredictable shift in weather conditions which caused both the recent Australian drought and the El Niño phenomenon with its profound economic consequences for South American fisheries and indeed, the whole of the South Pacific region. It is too soon to be sure, but the corals could be the first long-term record on which climate modellers can work.

Alun Anderson

Antarctica



Glaciology still on ice

SEEN from Australia, Antarctica is not a remote land at the uttermost end of the Earth but, rather, the nearest southern neighbour, closer to Sydney than either Singapore or Jakarta. Almost half of the continent — some eight million square kilometres — is claimed by Australia. And, uniquely, Australia's claim is disputed by no other nation.

For the time being territorial claims may matter rather less than does the smooth working of the Antarctic Treaty, which has kept the area demilitarized and unexploited and has, so far, provided a model of international scientific cooperation.

Keeping things this way is very much in the Australian interest. Militarization would expose Australia's southern flank, and changes in the environment could affect Australia's weather. Politicians have thus always stressed the importance of maintaining a visible presence there.

For Professor David Caro, vice-chancellor of Melbourne University, and for seven years chairman of the Antarctic Research Policy Advisory Committee (ARPAC), however, Antarctic visibility should mean only one thing — scientific research. But as things are, it seems there is just enough money to get men there but not enough for them to do very much. As he puts it, there has been a "credibility gap between what territory Australia claimed in the Antarctic and what it did there".

Professor Caro is about to end his chairmanship after seven years of what he calls "butting against a brick wall". But there have been some notable expenditures on the Antarctic effort in that period even though they have often been controversial. Sixty million dollars is being invested over ten years in rebuilding the three stations at Mawson, Casey and Davis — to the accompaniment of criticisms that the new designs are too expensive. As Minister of Science Barry Jones put it, "we have the best buildings in Antarctica, but we do not lead in much else". Building methods are now being reviewed and attempts made to cut costs by modular construction. More worrying is that the massive high-quality heavyweight buildings under construction will tie researchers to those particular locations for ever. A cheaper, less permanent solution might well have been preferable.

Construction should, in any case, speed up now that a five-year charter has been taken on a Norwegian supply ship *Icebird*. The new ship will also help one area where research has expanded and no complaints are heard — marine biology. Also in the "not too bad" category are cosmic-ray and meteorological research, which are continuing because they can be carried out

close to the bases. But it is a different story for glaciology and geology — areas in which Australia was once strong. The glaciology has petered out for lack of the tractors or helicopters to make this work possible.

A further bone of contention is that air strips are not maintained at the bases, so that senior scientists whose time is too limited to allow for the six-week voyage cannot fly to Antarctica. This is ironic as the Soviet Union is able to fly to its bases right in the middle of Australia's claim.

On the government side, the Antarctic Division of the Department of Science points out that overall spending has risen, although it has tended to be true that the government has accepted only the cheapest recommendations from ARPAC. But the government is proud of the political initiatives it has taken in Antarctica. The Convention on the Conservation of Antarctic Marine Living Resources came into force in 1982 largely through Australian efforts and is administered from Hobart. The convention is progressive in that it covers all living organisms in the Antarctic ecosystem. Management guidelines to ensure the ecosystem's stability are worked out by the scientific commission of the convention.

Australia is now active in promoting a similar convention on mineral resources; meetings of the treaty members have been taking place every six months. Progress has been steady, but there are potential problems. The parties to the Antarctic

Treaty consist of the seven original "claimant states", nine "full consultative" parties who have paid the heavy entry fee by setting up research facilities in the Antarctic and a further 16 "non-consultative signatories" who enjoy observer status at treaty meetings. Now the whole system has recently come under attack, particularly from Malaysia, which demands that Antarctica should become the "common heritage of mankind" rather than be run by nations rich enough to set up bases there. But conservation groups are concerned that a deal conducted behind the closed doors of the treaty meetings will permit some form of mineral exploitation, with concomitant risk to the environment; the fact that rich but resource-poor countries such as West Germany and Japan are increasing their Antarctic activity is seen as particularly suspicious.

It is in this climate that ARPAC has, time and time again, pointed out that "if Australia is to have an effective influence in mineral negotiations ... and make a significant contribution to the protection of Antarctica from any adverse effects of mineral exploitation", then it should fund research into the geology and related environmental factors of the Antarctic. So far these pleas have fallen on deaf ears. But there is now talk of setting up a new base, on the sliver of the Australian claim to the west of the Ross Sea, to complement the three sited further to the west.

Alun Anderson



Christchurch University's new campus lies among woodland on the banks of a tributary of the Avon.

sit back and "be highly selective about what you do". But this seems too much like making a virtue of necessity to appeal to the majority.

A dissenting view may also be heard from those who see the role of the universities somewhat differently. Dick Earle, professor of biotechnology at Massey University, says his main objective has been to establish a pool of well-trained graduates who could take biotechnology into industry, and in that he claims to be succeeding. He works closely with the frozen meat industry, but his research is not dull. He is working on the computer modelling of freezing objects with complicated shapes — the problem is one of phase change at a complex moving boundary, for which there is no analytic solution.

Many university researchers have made formal links with industry or the Department of Scientific and Industrial Research. Ten years ago, there was almost no contract work in universities: now engineers most noticeably, but also chemists and physicists, have taken on industrial work, chiefly because of financial pressure.

Underlying all the individual complaints made about the state of the universities is a sense of neglect. It is not just that there are problems, but that university scientists have, as one of them said, "no-one to wing to". Nobody really cares about them; nobody represents them at a level of government close to where decisions are made. Government, anyway, has often been singularly proud of its anti-intellectualism and has enjoyed making Aunt Sallies out of academics. But there are hopes that this will change as a body something like Britain's Science and Engineering Research Council may well emerge in the next year or so.

Within the present framework, there are varied opinions on the prospects for reform. One possibility would be to stop the too-even distribution of funds. A stiff dose of competitive peer review to replace the cosy "three wise men" might concentrate funds where they are needed and so concentrate the minds of those with real ability.

Professor Axford takes a more radical

Universities

Accessible but ailing science



THREE types of scientist can be distinguished in the New Zealand university research community: those who are furious about their current situation, those who profess philosophical resignation and those who are thinking of moving somewhere else. A fourth, and much rarer, species has found contentment in some external source of funds. Such was the impression gained when a group of scientists aired their frustrations at a small meeting at the Victoria University of Wellington. The essential problem is that there is so little money available that those who wish to do experimental science and to publish in international journals feel that the odds are against them.

One person who has decided to move elsewhere is Professor Ian Axford, vice-chancellor of the university. He is to return to a Max Planck Institute and feels deeply pessimistic about New Zealand's situation, particularly for young scientists, whom he describes as "totally demoralized". Conditions are indeed difficult and even British scientists would find them hard to bear. Funds available per student are 30 per cent less than in Britain and student/staff ratios are 50 per cent higher — which means enormous teaching loads.

But the main complaint is the virtual impossibility of buying a large piece of equipment. And as exchange rates fall, things are becoming critical for books and journals too. Wellington University's library is spending \$NZ1.5 million a year on its library out of the \$NZ25–30 million slice of the running grant it gets from the University Grants Committee. The fall in the real value of that money means fewer journals, and fewer delivered by airmail, which puts the rest of the world even further away.

This points to the second big problem — travel opportunities. It is best not to ask a group of New Zealand academics if they feel they have ample opportunities to visit world research centres. Sabbaticals are actually quite generous; leave can be taken just once every three years, and an air fare is provided as far as Perth (in Western Australia) and then an allowance (or pittance) of \$NZ20 a day. The only hope for improvement lies in increasing the number of international conferences being held in Australia. Faced with lack of funds and isolation, it is small wonder that some prefer the philosophic view and look at the advantages in being away from "the international hubbub", the opportunity to

Paying for the universities

RESPONSIBILITY for university finances rests with the University Grants Committee (UGC), whose main job is to assess the funds required to run the universities and to ensure that they are equitably distributed. Needs are assessed quinquennially and now amount to \$NZ200 million a year.

Division among the universities is determined by a formula related to student numbers, and is given as a block grant which can be spent as the university sees fit. Eighty per cent inevitably goes in salaries, much of the remainder on maintenance. What is left can be distributed to meet research costs. The system guarantees that all researchers are equally provided for in basic supplies, but it cannot provide large sums or expensive pieces of equipment.

To provide for equipment (that is, anything that an individual university cannot afford from its block grant, generally reckoned to be about \$NZ5,000) a separate UGC fund is available. But currently that stands at just \$NZ3 million a year, having just been hiked up from \$NZ1.8 million at the start of this quinquennium. Another \$NZ3 million a year is available for the replacement of equipment, and enough funds are provided to support 80 doctoral students a year. With possible claims on

the equipment money are some 623 "basic" researchers, 600 "applied" university scientists (including engineering, forestry and the like) as well as 314 doctors, dentists and vets.

The money is doled out not by independent peer review but "three wise men", who interview everyone who applies for a grant. Grant applications run at about three times the value of available funds. Mr David Hall, director of UGC, says, "we never really fund anything totally". It is expected that universities will always find some money elsewhere, by collaborating with the DSIR or otherwise.

New Zealand's seven universities are:

- University of Auckland
- University of Waikato (at Hamilton)
- Massey University (at Palmerston North)
- Victoria University of Wellington
- University of Canterbury (at Christchurch)
- University College of Agriculture (at Lincoln, near Christchurch)
- University of Otago (at Dunedin)

Auckland is by far the largest university with nearly 13,000 students. Canterbury, Otago, Wellington and Massey have 6,000–7,500 students each, Waikato slightly fewer than 4,000 and Lincoln 1,750. □

view. He believes that "basic science is withering" and, partly because New Zealand universities are too small, that success may "not be achievable in the existing framework". He proposes a single Institute of Advanced Studies responsible for "all postgraduate teaching and staffed with groups of more than critical size for strength and survival". Those working elsewhere, at what would become the equivalents of university colleges, would have to be given free time for study visits.

Despite all the problems, there remains one aspect of New Zealand universities

that greatly impresses outsiders: entrance to the universities is "open" for those over 21. At Wellington, 40 per cent of the students are over 25: it is not necessary to study full-time and grants are available to those who have not been to university before. Indeed, Wellington's oldest student is 82 and now doing a doctorate in classics after passing both BA and MA courses. As Professor Axford put it, "it is a very civilizing thing, which improves the whole tone of the community and stops intellectual fossilization". More of that might be welcome elsewhere. **Alun Anderson**

DSIR



Industrial disease

THE Department of Scientific and Industrial Research (DSIR) is the giant among New Zealand's research organizations: it consumes 45 per cent of the government's science budget and contains almost a thousand scientists, backed up by over a thousand technicians and support personnel, working in twenty-four divisions.

Virtually every area of science is covered — from oceanography and ecology to industrial processing and plant diseases — in what must be one of the world's last comprehensive government research bodies. Elsewhere, similar bodies have grown and fragmented; in New Zealand, the small size of the population and available resources has continued to make it essential to concentrate researchers in a few organizations so that there is some chance for them to reach a "critical mass" for efficient research.

Now, like equivalent organizations elsewhere, DSIR is under pressure to move closer to industry and to recover an ever-increasing part of its costs from the end-user. In 1985–86 its budget was to have been \$NZ97 million, and it will actually be \$NZ95 million; in the succeeding years it will fall to \$NZ90 million and then \$NZ82 million.

The director-general of DSIR, Jim Ellis, interviewed before these figures were announced but clearly having a good idea of what was coming, professed no pessimism about the future, however. At present, \$NZ13 million a year is raised from services, giving the DSIR a true budget of \$NZ110 million, and he believes that there are still hitherto sacrosanct areas, particularly in agriculture, where it would be fair to expect a bigger return from services.

Others within the DSIR divisions (see below) are very much more troubled about the effect the government cut-backs may have. Some too are much concerned about the increasing pressure to do research with short-term benefits. Jim Ellis himself characterizes a good half of DSIR's work as "very mundane, taking in the washing for a lot of government departments that don't have scientific facilities". That even extends to analysing alcohol levels in blood samples for the police. The rest is "long-term strategic research" intended to have application at some time in the future, plus cooperating on joint projects with industry.

But visits to DSIR research laboratories suggest it is not quite as simple as that. Anywhere you go will reveal lots of long-term, basic research going on, much of it no different from what would be found in a university, but rather well concealed behind the camouflage of an applied project. The strange thing is that it often



Buildings from the last century, now housing university departments, mingle with modern buildings on Auckland's campus. In the background, the science block.

DSIR consists of three main groups, each containing numerous divisions:

Primary production group

Applied Biochemistry
Crop Research Division
Entomology Division
Grasslands Division
Horticulture and Processing Division
Plant Diseases Division
Plant Physiology Division

Resources group

Botany Division
Ecology Division
New Zealand Geological Survey
Geophysics Division
New Zealand Oceanographic Institute
Science Information Division
Soil Bureau

Industrial Group

Applied Mathematics Division
Auckland Industrial Development Division
Chemistry Division
Christchurch Industrial Development Division
Industrial Processing Division
Institute of Nuclear Science
Physics and Engineering Laboratory
Wheat Research Institute
Information Technology Division

Separately administered are:

Antarctic Division
Geothermal Research Centre, Wairakei

looks as though it is the basic work that will, in the long-term, be much the more valuable.

Jim Ellis is also quite aware that lurking within DSIR are all sorts of odd characters with ideas of their own — and he is very proud of some of them. One story he tells is of the world's first natural gas-to-gasoline conversion plant, being built in New Zealand. A by-product of the zeolite conversion is a polycyclic aromatic called druedurene — which is a real nuisance because it crystallizes in carburettors and blocks them up. But now, thanks to one eccentric's work, it has been discovered

that druedurene can be turned into a high-impact strength polyimide plastic.

New Zealanders clearly value their individuality and around the labs one is often impressed by a neat solution someone has found to a problem — but usually to a rather small problem. In the DSIR's Physics and Engineering Laboratory (PEL), for example, a very neat little laser interferometer machine had been built that can automatically calibrate surveyors' staves at great speed. Much imaginative thought has gone into the work, but the gains might be greater if the same people were to be let loose on something bigger.

Another oddity perceived on a whistle-stop tour is that people are often quite unaware of the activities of other divisions — even when they are concerned with related disciplines. This is particularly curious in a country that is almost small enough for everyone to know one another and perhaps a result of the natural tendency of New Zealanders to be happier left to go their own way. But one gets the impression that for any given problem it could be quite hard to find just the right sort of person to help you. DSIR is certainly aware of this and with the Science Information Profile Systems (SIPS) being installed on the DSIR computer network is trying to remedy it. It will be possible to call up information on any number of staff or to find relevant personnel through keyword entries.

These information activities accompany a recent surge of activity in publishing glossy information pamphlets and advisory manuals for industry. Maybe before long they will be forced to stop telling the famous joke about DSIR: "What's the best way to run a small business? Buy a big one and ask the DSIR for advice".

Alun Anderson

Geology and geophysics

DSIR in the research field

GOD (or the gods that control earthquakes) perhaps sometimes works in favour of man. In 1855, just 15 years after Wellington was settled, New Zealand's mightiest earthquake on record tilted a block of land 30 miles wide and 120 miles long, lifting the shoreline at Wellington some five feet. The result was a nice level stretch of dry raised beach all along the coast. And that, local legend has it, allowed a fortune to be made by a settler who had been contracted to build a road along the shore and now found his work largely done.

The road, running just a few feet above the sea, now conveniently links Wellington, where DSIR's Geophysics Division has its headquarters, to Lower Hutt where the Geological Survey is located. Neither institute attempts the hopeless task of predicting earthquakes, which continue at an average rate of a magnitude 7 quake once in ten years and one of magnitude 8 once a

century. The Geophysics Division does, however, offer "words of wisdom and comfort", as one researcher put it, to a public worried about earthquakes and, more important, carries out seismic surveys at construction sites like dams so that, should the thing fall down, the contractor can at least wave the DSIR survey at the court of inquiry.

Accurate seismic risk evaluation, of course, depends upon good records and statistics of earthquake occurrence, and these the division has amassed in abundance. Sophisticated microprocessor-based monitoring systems have also been developed and will help automation of seismic networks. The division also maintains seismic stations in the South Pacific which forms part of the ocean's tsunami warning system. Altogether the division contains 35 scientists, backed up by an equal number of support staff, whose research covers the whole range of the Earth's natural



Forestry's fears

A MASSIVE transformation lies ahead for New Zealand's forest industry. At its heart is *Pinus radiatus*, introduced a century ago from California, where it is known as Monterey pine, but which grows phenomenally well in New Zealand. Planting is now at such a pace that, early next century, exports of forest products will probably have increased seven-fold, making forestry a major economic force.

The difficulties involved in this transformation occupy the minds of the 300 scientists and technicians of the Forest Research Institute (FRI), the research arm of the Forest Service. The switch to radiata pine is not the only change. Logging of indigenous forest has been virtually halted and the seven million hectares remaining must now be managed for recreation or as "protection" forest to prevent soil erosion or flooding. Just what the effects may be in a country prone to floods and landslides is one of FRI's problems.

The radiata pine is the most immediate challenge. Harvesting will be done in quite new kinds of areas, while the wood is of an unfamiliar type. Silvicultural regimes, which have a profound effect on wood quality, will have to be worked out by computer modelling of tree growth. New mechanical winches will be needed, while there are unknown dangers from pests. The quality of the wood means that new methods of cutting and processing must be developed. And so on.

FRI is facing these new challenges with enthusiasm but worries that the long-term research required in forestry will be hit by the demands of the government for short-term returns, despite the potential of the industry. Vital experiments on, say, how forests affect water resources and slope stability cannot be turned off and on, nor is there any end-user, other than government, to pay for them.

Yet FRI is better off than the universities, which FRI director Colin O'Loughlin describes as "poorly funded and getting worse". A serious problem, he says, is that "the anticipated expansion of forestry is not being catered for by the universities". So FRI must send people abroad for PhD training for lack of appropriate facilities in New Zealand.

Alun Anderson

dynamic activity. Much mapping has been done, including gravity mapping of the whole country, with an eye to the exploitation of natural resources of coal, minerals, oil and geothermal water. Parts of the survey services can be turned to a profit as can the hiring out of seismograph equipment to oil companies. This in turn helps areas such as volcanology that have nothing to offer industry. The pressure to recover more funds from industry sometimes places geophysics in an odd position: if a construction engineer wants to look at seismic records to help design a

safe structure should he be charged — if so, how much?

The Treasury desire that scientists should be looking for customers for their services also looks strange at the Geological Survey. The geology of New Zealand is extraordinarily complicated and the best way to understand it is through fundamental research. Trying to find out only things that someone might want to use would make progress impossible, nor would geological consultants appreciate it much if government geologists really began to compete with them for business!

The survey's 85 scientists perform the primary function of mapping as well as a range of research projects in petrology, palaeontology, Earth deformation and engineering geology. A major study is running on the Cretaceous and Cenozoic sedimentary rocks of the region, principally because they contain all the known coal and petroleum reserves. Most of the

basins of interest are offshore but the lack of an ocean survey vessel is not a problem — all the companies (mainly big foreign oil companies carrying out commercial survey in the region) are required to deposit data, cores and samples, and seismic tapes with the government. These came geology's way because the survey sells its services to the Ministry of Energy who need expert comment on applications for exploration licences.

Much of the expertise acquired in dealing with the petroleum industry is being passed on to smaller countries in the Pacific region. An invitation-only meeting has just been held on "Government and the petroleum industry" to advise representative geologists from the Pacific islands what to do when exploration companies come looking at the sedimentary basins, containing potential oil reserves, that some of the islands possess.

Alun Anderson

New Zealand's changing agriculture

A GLANCE at the histograms below will tell you that New Zealand's agriculture, responsible for some 80 per cent of export earnings, is in a period of transition. The traditional money earner — the export of meat — is scarcely growing at all.

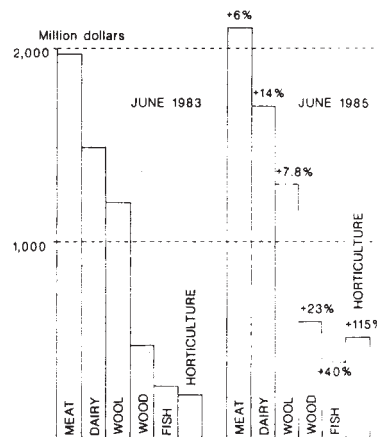
Intelligent diversification in the dairy industry has maintained some growth, and the demand for wool too is "holding up". But real growth is only being seen in fish, helped by the creation of the 200-mile protection zone; wood products, which are just at the beginning of a big leap forward; and horticulture, powered by the amazing success of kiwi fruit.

Just what has happened to New Zealand's traditional exports became clear from conversation with Pat Joyce, looking out from the roof of the Ruakura Agricultural Research Centre, Hamilton, over the acres of experimental land he is responsible for. In quite a few of those acres, deer are grazing in pens. An eager export market has been found for venison and the breed is being improved. Indeed, hunters who used to shoot wild deer from helicopters now bring them back alive for breeding.

The move to deer (along with angora and mohair goats) has come about because of the increasing difficulties of exporting the traditional products of sheep and beef. Most countries have quotas for beef now and, in what could be a big market for sheep, the United States, the Sheep Growers' Association form a powerful lobby. The traditional UK market was, of course, lost after Britain's entry into the European Economic Community.

With these changes comes an ever increasing need for research, firstly to exploit the new opportunities seen in the rapidly growing non-traditional product areas, and secondly to do something about the static areas. Despite the kiwi fruit

miracle, horticulture is not about to replace meat as the main export and new markets have to be found for the traditional products. Already there is much dependence on less-than-stable countries. Half of New Zealand's lamb now goes to Iran. China is taking a large part of the wool exports. But to break into the big US markets, changes have to be made — lamb, for example, would have to be made acceptable for TV dinners. At the same time, new spin-off products — such as adv-



anced milking machines — have to be sold overseas.

These demands come just as the government has announced that the 1985-86 budget for Ministry of Agriculture and Fisheries research is to be cut to \$NZ67 million from an expected \$NZ68 million; the next year it will fall to \$NZ63 million, and then to \$NZ58 million. The shortfall is to be recovered from industry by contract work and charitable services, such as soil analysis. This leaves places like Ruakura with a bit of a problem for they know, even if the Treasury does not, how vital it is to keep the long-term research projects going.

Alun Anderson

Information technology



DSIR in the picture

If all DSIR's scientists were put together they would not match the resources of even one big US or Japanese electronics manufacturer. What opportunities are there then for DSIR's newest division — that for information technology — set up, one hopes inappropriately, on April Fool's Day this year with just 20 scientific staff?

Surprisingly, the answer is "plenty", for the new division is finding a niche appropriate to its size. It has already equipped itself with glossy brochures from a software company that is selling an image analysis package developed by division scientists. And if the enthusiasm of its young director, Peter Ellis, continues to win over the government, New Zealand will soon have a chance to get in on the potentially lucrative satellite image processing market.

The image analysis package, called EPIC, is a flexible system for enhancing digitalized images — it can be used to deblur images, colour different image values, remove distortions, interpolate values and the like. There are a thousand and one uses for such packages, from intensifying details in X-ray images to picking out the number plate of a speeding motorist caught with a video camera.

But what particularly interests the new division is satellite image processing. Much of their expertise was gained in working on Landsat images (bought on large magnetic tapes) and their hope — inspired by some nods from the government — is that a ground station will be built to receive real-time images from SPOT, the French resources satellite to be launched later this year. Later on, other satellites could be tapped — the European Space Agency's ERS-I and then the two Japanese marine observation satellites. Use of SPOT would allow very fine mapping to be carried out as well as real-time crop analysis. Beyond that, if all goes well, there are even ambitions to participate in the design of instruments for use on-board spacecraft.

Most of the division's work is more down to earth. Major effort goes into running the DSIR's computer network and ensuring that the best software is available: here it is careful monitoring of what is available around the world rather than expenditure of original research that counts.

Image processing and the network between them absorb nearly all of the division's resources: what is left goes into projects such as a computerized system for the government printer, and character recognition systems to help with archiving in the National Library.

Alun Anderson

Advisory Council

A watchdog with no teeth

JUST as messengers carrying bad news run the risk of being blamed for the news itself, so central organizations for giving advice on science run the risk of being unpopular when finances are being cut.

New Zealand's National Research Advisory Council (NRAC) is no exception to this rule.

Nobody, it seems, has a good word for the council. The most charitable comment is that "NRAC is not what we need, but it's really not their fault". Indeed, if NRAC's recent record is anything to go by, it has laboured mightily with limited resources to advise a government which has little intention of listening. That may change, for NRAC is likely to be reborn in a new form. Almost nobody doubts that some such body is needed, and badly.

NRAC was set up in 1963 to give advice on the planning of scientific research, including the setting of priorities for government departments. Three kinds of obstacles have, according to government critics, made it impossible for NRAC to fulfil this role. First, the organization is very small (12 committee members) and relies on busy people, with commitments elsewhere, who cannot deal with the multitude of tasks that come their way. Second, it has never had a professional technical secretariat, but has to beg, borrow and steal personnel from the Department of Scientific and Industrial Research (DSIR) and other bodies. And finally, the government has tended to ignore it.

The line of communication to government is certainly long: NRAC reports to the Minister of Science (a low-ranking portfolio), who then reports to the cabinet, rather than directly to the Prime Minister. And again, the minister's portfolio includes responsibility for DSIR, so there is a confusion of his role as an overseer of the whole of science and as a director of one major science body.

To point out that there are shortcomings in the system is to say nothing new. NRAC's role has been under some kind of review for four years; self-criticism has been rife for longer. In July last year, the Probine report on the means by which the government obtains advice on science and technology policies recommended that NRAC should be revamped as a Science and Technology Council which, like those of other countries, would report directly to the Prime Minister. That report also asked for a new research council, with funds independent of those of the UGC, which could harness "under-utilized talent and equipment in the universities".

No action has yet been taken; NRAC is left "hanging in the air" as one member puts it, although action of some sort is still expected.

Uncertainty and criticism there may be, but NRAC has not been inactive. Its 1984

"Science and Technology Plan" surveyed the whole of New Zealand's research effort, and has not escaped criticism. Those involved in applied research say that it states the obvious, while those in the universities are offended that "The role of the universities" occupies a mere two of 166 pages. But the report was intended to be "more of a review than a plan"; a second plan is to appear shortly and seems considerably more vigorous.

To compile the 1985 plan, the "users" of science (manufacturers and primary producers) were approached to discover their needs; merely making this effort seems to have awakened considerable interest among the producers. Scientists also had an opportunity to air their views at a large May conference on "Science and Technology for Development", which generated several inches of documents from indus-

trialists, technologists and academics on the remedies for New Zealand's woes. No panaceas or plans for radical reform emerged, but plenty of heated argument.

The head of NRAC, Alan Mackney, is not averse to expressing a few strong opinions either. On the government's attempts to recover a fixed percentage of funds by the user-pays principle, he says that "an arbitrary amount to be got without rhyme or reason" just does not work", and that if the user, who often does not know what he needs, is to decide what must be done, New Zealand will be "frozen into pre-technology".

The annual reports of NRAC, too, contain endless recommendations that science should be taken more seriously. But until NRAC is reborn as a body that represents all scientists and technologists (and the universities have been conspicuous by their absence) and until it has real teeth (access to decision-makers and money), its activities will continue to be all bark and no bite.

Alun Anderson



Geothermal energy

Looking for a crock of gold



NEW Zealand can proudly point to one area of research where it is a world leader: the chemistry and physics of geothermal systems. Confounding the politicians who are now demanding a short-term pay off from science, the long-term accumulation of expertise in geothermal research has allowed two New Zealand consulting firms to win big foreign contracts for the design of geothermal power plants. In 1958, New Zealand became the first country in the world to build a geothermal power plant operating on the mixture of steam and hot water that is found in the vast majority of the world's geothermal fields. Part of the steam was originally intended for a UK heavy-water project never carried through. Fortunately, the flexibility of the design for turbine operation has meant that the power plant, at Wairakei, is still operating with utter reliability producing 5 per cent (160 MW) of the country's electricity.

To generate the electricity, one tonne of fluid a second is taken from boreholes drilled one to two kilometres into the ground. The area has become a mecca of geothermal understanding. There have been some surprises. As steam has been taken out of the ground, the water table has fallen as much as 15km away, and the surface has subsided, in one place by 0.4m a year. A nearby tourist attraction that used to be called Geyser Valley is now known as Geothermal Valley because the geysers have turned into mud pools. On top of that, an unhealthy level of arsenic has been found in the geothermal water discharged into the river that flows down to the town of Hamilton.

None of these problems has proved as serious as might appear, however, while the expertise gained and the basic research done on the geothermal system have paid dividends. Now two geothermal companies, GENZL and KRTA, contain-



An active geothermal field in central New Zealand.

ing many researchers formerly with the Department of Scientific and Industrial Research and the Ministry of Works, have taken a large share of contracts in the Philippines, which is now second only to the United States in the number of its geothermal power plants.

The latest surprise at the Geothermal Research Institute has been for the geochemists: the insides of some of the borehole pipes have been found to be coated with gold. What seems to be happening is that, in the geothermal system, gold forms a complex with dissolved hydrogen sulphide which precipitates when the pressure drops. The implications are important. Hitherto the amount of gold deep in the system has been inferred from that left in the geothermal water, but with the amount now known to be deposited added to that, it seems that, deep down, the hydrothermal water is close to saturated with gold. This strengthens the case for thinking that the geothermal system beneath Wairakei is a living model of fossil geothermal systems that are now being actively mined for gold and study could thus aid prospectors. The work on gold deposition is not yet published, but Richard Henley's book *Fluid-Mineral Equilibria in Hydrothermal Systems* is already a modest bestseller, going into a second printing after nine months.



Capable of supplying 5 per cent of New Zealand's electricity, the Wairakei geothermal power plant.

While prospecting for gold may be in fashion, prospecting for steam is in the doldrums in New Zealand. The black art of prospecting has been refined by the Wairakei centre so that the chances of a

borehole turning out to be successful are very high indeed. The technique is a combined operation between geologists, who do the basic mapping, geophysicists, who use electrical resistivity and seismic techniques to map the reservoir, and geochemists who reckon to predict subsurface temperatures from analysis of fumarole steam. (Asking which group contributes most is a sure way to begin a brawl.) But there is little chance that new power plants will be built in New Zealand. Instead, the tricks of the trade are being transferred to others at a special Geothermal Institute, supported by the United Nations, at Auckland University.

Just why New Zealand has been able to keep the lead in this area was neatly explained by Jeff Hedenquist, a geochemist at Wairakei, and himself from the United States. In New Zealand, all geothermal work is done by government agencies and information is freely available; in the United States, by contrast, there is free competition for contracts which sometimes means that one system is drilled by several companies without any sharing of information. The result is that it may be difficult to understand the system as a whole. Perhaps the New Zealand government should bear this in mind before pushing ahead with its plans for "liberalization".

Alun Anderson

Chinese gooseberries and tree tomatoes lead the way

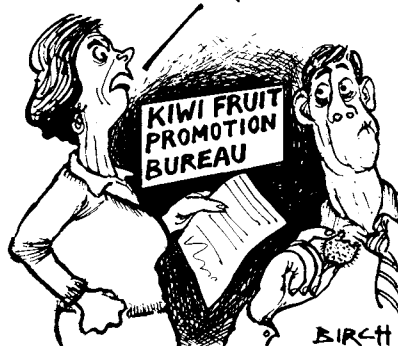
"It's a miracle", said one Australian agriculturalist, "that the New Zealanders have been able to sell that horrible looking, tasteless fruit". He was uncharitably referring to the kiwi fruit, a few years ago totally unknown and now on supermarket shelves from Tokyo to London, this year worth \$NZ220 million in export sales.

The curious thing about the kiwi fruit is that it really has nothing at all to do with New Zealand. Its first mention is in a poem written in the Tang dynasty: the kiwi fruit is Chinese, and came to New Zealand only by chance. At the turn of the century, in Yiohong, on the Yangtze River, a small colony of missionaries and traders learned to cultivate the fruit as a local novelty. Among the missionaries were three young Church of Scotland ladies from New Zealand. One of them had an adventurous sister who came out to China for a holiday, and took back with her seeds of the "Chinese gooseberry" which she passed on to a local sheep farmer with an interest in novel plants. All the kiwi plants in New Zealand can be traced back to these first specimens.

As the Chinese gooseberry, the plant was never a commercial success. Turners and Growers, New Zealand's best known fruit merchants, tried to sell it in California without success. At a meeting called to remedy matters the chairman of the board, in a moment of inspiration, rejected a suggestion that the fruit be renamed the

"melonette" and pronounced, "this is the land of the kiwi and it shall be the kiwi fruit". The rest is history. Exports have grown from \$NZ4 million in 1976 to \$NZ220 million this year. From the number of kiwi fruit plants now going into the ground, it is predicted that by 1990, the industry will be worth \$NZ740 million. If that happens, horticulture will account for 13 per cent of New Zealand's exports com-

It's from the U.S. Navy—can we sell the idea of nuclear warships?



pared with 5 per cent now and just 2 per cent five years ago.

So Rod Bielecki, director of the DSIR Division of Horticulture and Processing, wonders if the government knows exactly what it is doing. The Treasury is squeezing research funds and demanding that more money be recovered from industry, just as

an important new export sector is being born. Behind the kiwi come a host of other exotic fruits being readied for the export market: fijoas, tamarillos (no longer called "tree tomatoes"), persimmons, Asian pears and pepinos, already not uncommon in Tokyo supermarkets.

To make them into real winners, there will have to be selection programmes to improve taste, cultivars specially suited for New Zealand conditions, research on plant pests and on the improvement of keeping qualities. Bielecki feels the government is far too keen to transfer the burden of research to industry when, if anything, increased government research in this sector would pay dividends.

To those who say that the money should be recovered from the farmer, Bielecki replies that most horticultural end-users are small single farmers, who need one central research institute. Nor does Bielecki believe the government understands the role science plays in generating wealth. It was one of the division's predecessors which identified kiwi fruit varieties that keep long enough for them to be exportable. More recently, the division was called in when an unexpected frost hit before harvesting was complete, and it seemed that much of the crop might have to be dumped. As fruit growers expand into less favourable land, and as the range of fruits increases, there will be many other problems to solve.

Alun Anderson

Stefan Marinov wins friends

The suggestion that there are systematic departures from the strict requirements of special relativity has been persistently put forward by Dr Stefan Marinov. There is a case for repeating his experiment.

STEFAN MARINOV is a remarkable iconoclast who is convinced that Einstein's special theory of relativity is mistaken. Bulgarian by origin, Marinov has been, for all practical purposes, exiled from his homeland, but it is by no means clear whether his opinions on relativity or on the proper conduct of the Soviet leadership are chiefly responsible.

From his new home in neutral Austria, Marinov has been at loggerheads with several scientific journals, *Nature* among them, because of their refusal to publish any but a small part of his work, which now also includes a purported demonstration of a *perpetuum mobile*.

Marinov's frustrations with the journals have recently seemed to get the better of his judgement. Last year, angered by *Nature's* refusal to publish a group of manuscripts, Marinov was for a time threatening to immolate himself in the square outside the British consulate at Genoa. Later, he threatened to do the same outside the British embassy in Vienna, but fortunately settled for a press conference instead. Those who have had dealings with Dr Marinov can have been left in little doubt that he is passionately convinced of the correctness of his position on special relativity. Quite apart from his apparent willingness to take his own life, it is clear that his waking hours are almost all spent in correspondence, much of it unfortunately fruitless, about this theory and about his purported demonstration, by means of what he calls the "coupled-shutters experiment", that the velocity of light is not, as Einstein's theory supposes that it would be, equal in all directions (isotropic) in all frames of reference.

Now, it seems, Marinov has won some influential friends. The issue of *Physical Review Letters* for 8 July (55, 143; 1985) contains an article by A.K. Maciel and J. Tiomno from the Brazilian National Science and Technology Research Council at Rio de Janeiro which at least puts Marinov's objections to special relativity in a context in which they can be grappled with. Moreover, what Maciel and Tiomno say is certain to have an important bearing on the classification of all possible experimental tests of special relativity.

Plainly, when there is such a mountain of experimental evidence that the general features of special relativity are borne out in practice, it would be foolish in the extreme to assert that special relativity is just plain wrong, thereby implying that the

time has come to return to newtonian mechanics; after all, objects do not travel faster than light in any circumstances observable, while time dilation (as defined by Lorentz) is a fact of life and can be measured, for example, by the difference between the average lifetime of an unstable particle in motion and at rest. So if there are violations of special relativity, they are at most weak violations, departures from what is not strict orthodoxy and not flat contradictions of it.

But what weak violations of special relativity are neither trivial nor absurd? Borrowing from some work due to H.B. Ives in the 1930s, Maciel and Tiomno argue that it is prudent to disallow violations for electromagnetism, for independent particles in uniform motion and the other more elementary phenomena used as illustrations of special relativity, now even in the elementary textbooks. But what about the possibility that the behaviour of rigid bodies is not accurately accounted for by the Einstein-Lorentz transformations of coordinates, and that a rigid body remains rigid — the separation of fixed points remains constant — however it is moving?

The formal realization of this state of affairs seems not particularly elusive, although it is not possible to modify special relativity in any meaningful way without reintroducing the newtonian concept of absolute time and space, or the idea that there may be some preferred frame of reference such as that of the microwave background. Explicitly, if x, y and z are cartesian coordinates in that absolute frame of reference, and t is the time, and if primed quantities are the same coordinates in a system moving at velocity v along the x -axis, the transformed coordinates are exactly those of special relativity except for t' , which is given (according to the formalism of Maciel and Tiomno) by $t = \gamma t'$, where $\gamma = (1 - v^2/c^2)^{-1/2}$ and not the conventional Lorentz transformation in which xv/c^2 is added to t' .

The calculations are not in themselves particularly difficult. It emerges simply enough that rigid rotating bodies remain rigid in the new system, and in the particular sense that the apparent angular velocity of all fixed points is a constant (if the angular velocity of the whole object is constant). The velocity of light is isotropic in the absolute system against whose coordinates the rotation of the rigid object is defined, while both time dilation and

the Lorentz-Fitzgerald contraction (of lengths in the direction of relative motion) persist. What is sacrificed is the isotropy of the velocity of light in the rotating system.

The interest of what Maciel and Tiomno have done is that they can sort the several published tests of special relativity by the likelihood that they will yield a stringent test of the weakly violated form of special relativity defined by their transformation. The classical test of special relativity first carried out by Michelson and Morley and since repeated several times does not meet the test. The version of the experiment due to Joos (published in 1930) in which a Michelson interferometer was rotated on its turntable once every ten minutes or so, classically one of the better verifications of the isotropy of the velocity of light (among two orthogonal directions), will not suffice, although a more rapidly rotating turntable might meet the need. The simulation of the Michelson-Morley experiment in which two hydrogen masers are used as the source of radiation moving in orthogonal directions offers more hope of providing a stringent test of the modified transformation equations. And so, Maciel and Tiomno say, does Marinov's rotating-shutters experiment.

The principle of that is simple enough, a kind of Fizeau measurement of the velocity of light except that the objective is to make a direct comparison of the velocity of light in two opposite directions. Two disks with identical perforations are mounted parallel to each other at opposite ends of an axle and light from a laser is directed (by means of a beam-splitter) through the perforations of the disks in each direction. Marinov claims that his results, most recently obtained with home-made equipment at Graz, demonstrate that the velocity of light is not the same in all directions. He even claims to have been able to detect the velocity and direction of the Earth's movement through absolute space and time.

None of this proves that there is anything wrong with special relativity. It is merely a pointer to the kinds of tests that would be necessary to demonstrate a particular (and "weak") violation thereof. Maciel and Tiomno merely say of the Marinov experiment (as of the others on their list) that it "should be repeated even if to prove it wrong". It will be interesting to see how many correspondents write in to claim that that has been done already.

John Maddox

Haemoglobin structure

A clam with a difference

from Max Perutz

ALL vertebrate haemoglobins are tetramers made up of two pairs of unlike polypeptide chains, termed alpha and beta, each chain forming a basket for one haem. But on page 227 of this issue W. E. Royer Jr, W. E. Love and F. F. Fenderson demonstrate that haemoglobin of the clam *Scapharca inaequalvis* has a quite different form of subunit assembly.

In vertebrate haemoglobins, the alpha chain is coiled into seven helical and six non-helical segments, and the beta-chain is coiled into eight helical and six non-helical segments. The tertiary structures of both the alpha and beta chains are similar to each other and to myoglobin, and the residues lining the haem pockets are largely homologous. The vertebrate tetramer has pseudo-tetrahedral symmetry with one true dyad axis running through a central water-filled cavity and two pseudo dyads at right angles to each other and to the true dyad. Assembly occurs in two stages: first, one alpha and one beta chains form a tightly bound dimer; and second, two such dimers combine to form a tetramer. The allosteric change between deoxyhaemoglobin and oxyhaemoglobin consists in a large rotation and slight translation of the two alpha-beta dimers relative to each other.

The haemoglobins of two primitive vertebrates, lampreys and lungfish, are monomeric when oxygenated and oligomeric when deoxygenated; the monomer has a tertiary structure similar to myoglobin. The same is true for all the invertebrate haemoglobins whose structure has been determined and for the haemoglobin found in the root nodules of leguminous plants, suggesting that the myoglobin fold is a universal pattern (see figure) that has evolved to provide the surround for an oxygen-carrying haem.

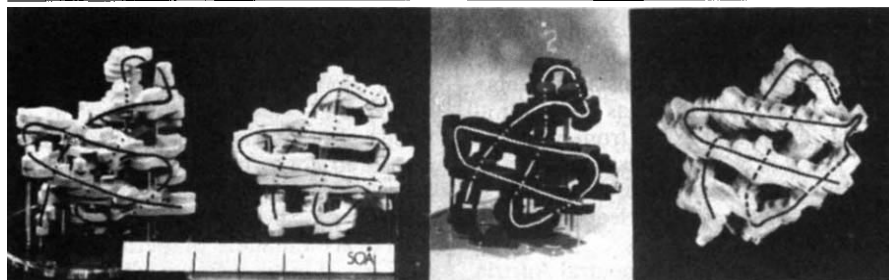
A suspicion that the quaternary structure of invertebrate haemoglobins may be different from that of vertebrates arose from the observation that one of the larval haemoglobins (component X) of the fly *Chironomus thummi thummi* has a marked Bohr effect coupled to a dimeric structure at pH 5.5 and to a monomeric structure at pH 7.0. Bohr effects probably arise from the ionization of histidines, but no histidine in this haemoglobin is at a position corresponding to subunit contacts in vertebrate haemoglobins.

Royer *et al.* now show that haemoglobin of the blood clam *S. inaequalvis* has a tertiary structure similar to myoglobin, but with one additional helix at the amino end which lies parallel to the carboxy-terminal helix H and brings the terminal amino group close to the terminal carboxy group. It is in its quaternary structure that

the clam haemoglobin is exceptional. Two like monomers assemble to form dimers, not by contact between helices G and H as in vertebrates, but by contact between the

between helices A, and between the non-helical segments NA and GH. The dyad axes of each of the individual dimers make angles of 75° with the dyad joining the two dimers, so that there is no tetrahedral symmetry.

The structure solved is that of the carbonmonoxy-derivative which is probably isomorphous with the oxy-derivative. Oxygen binding is cooperative with a Hills coefficient of 2.1, and loss of



The universal tertiary structure of, from left to right, sperm-whale myoglobin, horse haemoglobin alpha chains, horse haemoglobin beta chain and haemoglobin of the clam *Glycera dibranchiata*. In each picture the amino end is on the left.

haem-linked helices E and F, so that the haem pockets lie on the inside of the tetramer, as Pauling once imagined, rather than on the outside, as in vertebrates. Two chemically different dimers join to form a tetramer; as far as one can make out from Fig. 2 of Royer *et al.*, the tetramer is formed by contact

oxygen is linked with polymerization of the tetramer. It will be interesting to see if solution of the deoxy-structure reveals the mechanism that triggers this polymerization. □

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X-ray astronomy

A new breed of oscillator

from N. E. White

THE first short timescale periodic oscillations from a bright galactic centre X-ray source (GX5-1) are reported by van der Klis *et al.* on page 225 of this issue. These quasi-periodic oscillations (QPOs) probably indicate the presence of an accreting neutron star that is rotating with a period of a few milliseconds. This discovery may provide timely support for recent models for the evolution of low-mass X-ray binary stars (LMXBs) into millisecond radio pulsars. Furthermore, the oscillations themselves display intriguing characteristics requiring some ingenuity from theorists seeking to explain them.

An accreting neutron star will only be seen as an X-ray pulsar if its surface magnetic-field strength is high enough to tunnel the inflowing material from its companion star onto the magnetic pole. The spin-down properties of radio pulsars suggest that neutron star magnetic fields decay on a timescale of 10^6 – 10^7 years. If neutron stars are born with the same surface field of 10^{12} – 10^{13} Gauss, then those located in the X-ray binaries should show a range of field strengths depending on their age. X-ray pulsars are usually associated with young population I objects (OB stars). In contrast, the older population II

LMXRB systems (where the mass-losing star is of a late special type) rarely contain X-ray pulsars.

Any material that flows between components in a binary system must lose angular momentum before it can fall onto a compact object. It may form an accretion disk which will mediate the flow and, by exerting torque, will spin the neutron star up to equilibrium period at which its rotation period is equal to that of the innermost keplerian period of the disk. If a strong magnetic field is present this is determined by the radius at which the magnetic pressure disrupts the disk; for a typical X-ray pulsar with a field of $\sim 10^{12}$ Gauss the equilibrium period is a few seconds. If the field is less than 10^8 Gauss this disk will extend to the neutron star surface and the equilibrium period is ~ 1 ms. It takes only 10^6 – 10^7 years to spin a neutron star up to this period.

This scenario has encouraged the search for millisecond periodicities from LMXRBs but with no success until van der Klis *et al.*, using the European Space Agency X-ray observatory (EXOSAT) because it is probably more sensitive to millisecond periodicities than previous X-ray satellites, searched again for milli-

second pulsations from the brightest of the LMXXRB — the galactic bulge sources.

The oscillations found from GX5-1 are not at a single frequency but rather show a distribution of frequencies whose centroid and width are correlated with luminosity. An increase in luminosity by a factor of only 1.4 causes the average frequency to increase from 20 to 40 Hertz and the frequency spread to increase from 4 to 12 Hertz.

The large variation in frequency eliminates the possibility that QPOs directly reflect the spin period of a neutron star. Any model must explain the very strong dependence of oscillation frequency with luminosity. Of the various possibilities discussed to date, the most promising seems to be an instability at the edge of a magnetosphere. Magnetospheric models use the fact that an increase in mass-transfer rate (luminosity) will cause a compression of the magnetosphere such that the characteristic keplerian period at the inner edge of the accretion disk becomes shorter. This gives a frequency/luminosity dependence that is in the correct sense and, with some refinement described elsewhere in this issue², the correct magnitude. These models predict a neutron star rotation period of roughly 10 milliseconds and a surface magnetic field in the order of 10^9 Gauss for GX5-1.

The orbital periods of high-luminosity LMXXRBs ($\sim 10^{38}$ erg s⁻¹) such as GX5-1 are in general unknown, but in the two sources where they have been measured, ScoX-1 and CygX-2, are found to be 0.7 and 9 days. In these cases, only an evolved giant companion star can fill its Roche lobe, such that the mass transfer will be driven by the evolutionary expansion of the giant (causing the stars gradually to spiral apart). In such a system the mass transfer ceases when the mass-losing star becomes degenerate.

It has been pointed out that the compact object may begin as a white dwarf, but because of the extra mass accreted, is pushed over the Chandrasekhar limit to collapse into a neutron star³. This could lead to a young X-ray pulsar in an LMXXRB. The magnetic field then decays on a timescale that is a factor of ten shorter than the evolutionary timescale. It is plausible that if the neutron star is formed close to the end of the mass-transfer phase then a sizable magnetic field will be left and a millisecond radio pulsar born.

The discovery last year of a 6-millisecond pulsar that has a degenerate companion in a 120-day orbit caused much excitement because such a binary is expected as the end-point of this scenario⁴. The parameters deduced from the magnetosphere model for the underlying neutron star in GX5-1 are similar to those determined for the 6-millisecond radio pulsar. This may be a coincidence — if the 6-millisecond pulsar evolved from a LMXXRB it would presumably have looked very like GX5-1 does today.

The magnetosphere model, however, has at least two worrying aspects. First, it is perturbing that the underlying rotation period is not detected. Although there may be ways around this problem (for example, smearing of the pulses in surrounding material) it remains unsolved. Second, the nature of the magnetosphere model allows it to reproduce any frequency/luminosity relation if the dynamic range of the luminosity variation is small. This makes the model extremely flexible, but means that it is not uniquely specified by the data. Other possible models for QPOs are not sufficiently developed to make specific predictions. One suggestion (based on a similar phenomenon that has long been known from the cataclysmic variables) is that the neutron star does not have a substantial magnetosphere, and that the oscillations are generated in the large velocity gradient of

the boundary layer between the disk and the neutron star surface⁵.

Following the GX5-1 result, QPOs have been found in two other low mass X-ray binary stars of high luminosity and long orbital period, ScoX-1 (ref.6) and CygX-2 (ref.7). QPOs had previously been missed in these two sources because observers were searching for single peaks in their power spectra rather than broad humps. □

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Archaeology

Industrial revolution in York

from Richard Hodges

RESCUE archaeology has produced a wealth of information about town and country settlements in Britain from Neolithic to modern times¹. These archives constitute a remarkable documentation of our heritage. However, in a phase of stock-taking imposed by current financial constraints it seems appropriate to ask how we have benefited from these excavations.

Most rescue excavations have been concerned with historical archaeology. Redevelopment of inner city centres has allowed deep-stratified excavations of medieval, Anglo-Saxon and Roman layers. The same has happened in the countryside where deep ploughing has obliterated very many remains of Roman-period, Anglo-Saxon and medieval villages and farms. One consequence of this destruction is that there now exists an accurate measure of all these types of historical settlements. The prospects of writing a history based on material remains

has not been fully appreciated until now. To illustrate this point I discuss here some recent excavations which reveal what the written sources do not — the first English industrial revolution.

Rescue excavations reveal the scale of change that occurred in about AD 900. The

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Excavations at the Coppergate dig, York. (Courtesy of Jorvik Viking Centre, Coppergate, York YO1 1NT, UK).

Viking dig at Coppergate, York (see figure) provides probably the most perfect illustration of the industrial explosion which occurred at the beginning of the tenth century². Rescue excavations beneath the modern tenements at Coppergate have uncovered a collection of workshops laid out at right angles to the modern street grid. The timbers surviving in these structures are dated by dendrochronology to about AD 900. Much rubbish, associated with the occupation of the associated tenements as well as their workshops, provides some measure of the revolution in craft production in Viking York. As Richard Hall shows², these tenements were planned on largely unoccupied ground; coopers, moneyers and smiths worked here in the tenth century. Botanical evidence as well as the microscopic remains of beetles and other insects betray the living conditions of the new urban class, whereas excavations elsewhere in the city show a high mortality rate among children and a shorter life-span for women by comparison with rural standards of the age³.

Coppergate, like many smaller rescue excavations in York, confirms the decline of the Roman town in the fourth century, as well as the general desertion of the centre between about AD 400 and 900. The urban take-off, focused on these earlier administrative settlements, occurred in around AD 900, and with it York was transformed. Rescue excavations in Viking towns such as Colchester⁴, Lincoln⁵, Northampton⁶ and Thetford⁷ have documented the transformation from a rural elite centre to a vibrant market town during this period. At Northampton, for example, the middle Saxon minster and the Mercian palace have been located and excavated; both were subsumed into the matrix of a fast-expanding tenth century market⁸. Indeed, the mark of the Viking age in eastern England are these markets and the development of craft production.

New markets are also a prominent feature of most southern English towns at this time. Their foundation may be considered the real achievement of King Alfred the Great. Recent excavations in Canterbury, Chichester, Exeter and London⁹, as well as Martin Biddle's famous excavations in Winchester¹⁰, all conclusively demonstrate that these places had lain more or less vacant since Roman times until they explode into life once more at the end of the ninth century. Most medieval street systems stem from this time. Thousands of tons of gravel were quarried and laid in an operation that was as complex as any by Roman land surveyors. Decaying fortifications were refurbished, new tenements were designed and, as in York, craftsmen were starting in business almost overnight, producing a great variety of commodities for the surrounding rural markets. Alfred's kingdom was on its way from being an underdeveloped economy to one which, seventy years later, was one

of the richest in western Europe.

The industrial revolution was necessarily supported by changes of comparable scale in the countryside. Rescue excavations at Raunds in Northamptonshire¹¹ have charted the emergence of the medieval village, and it is a pattern now noted in many other places. The middle Saxon farm was evidently transformed between about AD 850 and 950, when it became part of a collection of farms focused for the first time on a manor and a parish church. Indeed, the manor and parish church seem to be features of the revolution as local lords and the Church tried directly to control agrarian resources. In the tenth century the number of rural communities expanded rapidly.

The scale of this revolution is mainly a measure of late Saxon and Anglo-Scandinavian government. Powerful centralized kingship was a feature of England from the Viking age to the Norman conquest and in complete contrast to the diffused authority of most continental kings¹². The authority and the capacity to introduce markets and the market principle to Anglo-Saxon England is a measure of political control¹³; to execute it on the scale witnessed in these excavations is a measure of the nation's wealth at that time.

A generation of rescue archaeology, therefore, has shed light on a momentous movement. With the agrarian and industrial revolutions of Alfred's age the form of present-day landscapes and townscapes in

Britain took shape. But archaeology on this scale offers more than a chance to reassess local history; it makes it feasible to calibrate the management of resources and to examine the social implications of such strategies. It is certain that the identification of this first English industrial revolution is just one example of the opportunities to rewrite the history of the Roman and later medieval periods. Rescue archaeology has convincingly shown that mainstream history can be studied in the field as well as in the archive. □

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Malaria vaccine

Components of a cocktail

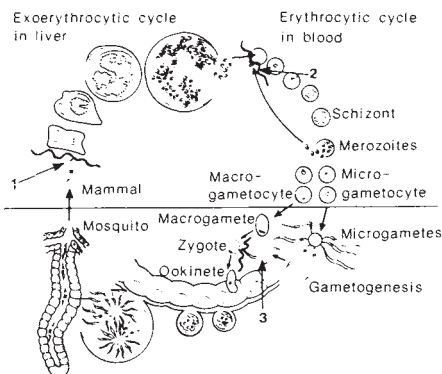
from F.E.G. Cox

IN THE wake of spreading drug resistance, the search for a realistic malaria vaccine has taken on a new urgency — most workers now agree that this will have to consist of a 'cocktail' of antigens or, as the World Health Organization puts it, a multivalent vaccine. Rapid progress has been made in identifying and characterizing potentially protective antigens from malaria parasites at various stages of their life cycle. A major antigen of the male gamete of the rodent parasite, *Plasmodium yoelii*, which can elicit antibodies that block transmission of the gamete from mice back to the mosquito vector, is described on page 258 of this issue¹. The human equivalent of this antigen may eventually be an important constituent of a vaccine but needs to be considered in the context of all the other antigens contending for this final selection.

The complex life cycle of the malaria parasite (see figure) begins when sporozoites are injected directly into the bloodstream by a mosquito. The sporozoite is, therefore, an obvious target for immunization and the major sporozoite antigen of the human malaria parasite,

Plasmodium falciparum, has already been expressed in *Escherichia coli*² and has proven immunogenicity³. Equivalent antigens have been used to immunize laboratory animals with considerable success. Unfortunately sporozoites circulate for only a few minutes before entering liver cells where they undergo a massive phase of multiplication and acquire new antigenic specificity. The products of multiplication, merozoites, which are again antigenically distinct, invade red blood cells to initiate the most destructive multiplicative phase of the life cycle.

Relatively little is known about the stages preceding this initial red-cell invasion but the intraerythrocytic stages have been intensively studied. Many antigens have been detected and the potentially important ones appear on the surface of the dividing parasite (schizont) at a late stage and on the merozoites resulting from division. These antigens, which vary from species to species and even within species, have relative molecular masses (M_s) between 190,000 (19K) and 23K (ref.4). Purified and partially purified antigens have been used with some suc-



The life cycle of *Plasmodium* spp. in mammals. The main targets for the immunological control of malaria are: (1) anti-sporozoite (prevents entry into liver); (2) anti-merozoite (prevents entry into red blood cell); (3) anti-gamete (prevents fertilization). Wavy line indicates blocking. Redrawn from Cox, F. E. G. *Modern Parasitology* (Blackwell, Oxford, 1982).

cess to immunize laboratory animals; the protection seems to involve blocking attachment and invasion by the merozoites of fresh red cells. The merozoites themselves possess several distinct antigens associated with either the surface or internal organelles. The gene for at least one of these surface molecules has been cloned and expressed in *E. coli*⁵ and a surface antigen has been used to immunize monkeys with partial success⁶. Some of these antigens may be involved in attachment of the merozoites to the red cells.

The final stage of the life cycle begins when merozoites enter red blood cells and develop into sexual stages or gametocytes. These remain in the red cells while in the blood and possess yet different antigens. Normally there is no immune response against the gametocytes, but once they re-enter a mosquito, they leave their protective red cells as gametes and become vulnerable to any antibody that is also taken up with the blood meal. These are the stages studied by P. G. Harte, N. Rogers and G. A. T. Targett¹. Using a rodent model, *P. yoelii*, they have identified on the male gamete a major antigen with *M*_{42K} and several minor antigens of 67, 59, 57 and 35K. Mice were vaccinated with these purified antigens and later infected with *P. yoelii*. The infections progressed as normal and gametocytes formed. However, when mosquitoes were fed on the mice they failed to become infected. The mechanism seems to involve agglutination of the male gametes and the prevention of fertilization. The effects of immunization were dramatic; the parasite load in the mosquitoes was reduced by 99 per cent following two immunizations.

There is every reason to expect that this kind of vaccination will be successful in primates, particularly as earlier work with crude antigens has already been confirmed in monkeys. These antigens, however, are not the only ones associated with the gametocytes and gametes; antibodies against the other antigens block

various stages of development and one even reduces the number of gametocytes in the blood¹.

The real problem with human gametocyte or gamete vaccines is that they are specific to these stages and do not affect the course of the infection. This means that, for ethical reasons, they could not be used on their own — hence the need for a cocktail of antigens that would at least ameliorate the progress of the disease in man. Another problem is that there is still no rigorous mathematical analysis of the efficacy of such vaccines in the reduction of malaria transmission, so we really have little idea about how effective this method of preventing transmission might be.

There are problems other than the ethical ones. Although the emphasis has

been on antigens and antibodies it is clear that antibody-independent mechanisms are also involved in immunity to malaria⁷. At one time it was thought that a sporozoite, a merozoite and a gametocyte antigen mixture might do the trick but it now clear that the cocktail must be very complex indeed. □

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Mathematics

The Bierberbach gambit

from Ian Stewart

THE theory of complex functions was the centrepiece of nineteenth century mathematics and is still of fundamental importance. It has been worked over thoroughly by generations of mathematicians, and one might imagine that everything worth knowing is now known; but in fact many outstanding problems remain. Among them was a conjecture in geometric function theory made by Ludwig Bierberbach in 1916, which bounds the coefficients in the Taylor series of a complex analytical function satisfying certain conditions.

Despite a sustained attack, the conjecture was verified only in a few special cases until last year when Louis de Branges obtained a complete proof, now available in preprint form (de Branges, L. *A Proof of the Bierberbach Conjecture* Mathematics Department, Purdue University). His method is a development of one used with partial success by Karl Löwner in 1923, and the final gap is filled by an inequality proved by Richard Askey and George Gasper in 1976, in the apparently unrelated (and unfashionable) field of special functions. Initially, de Branges's claim was greeted with scepticism. The tale casts an interesting sidelight on the sociology of mathematical research, as well as its content.

Geometric function theory, as its name suggests, deals with the geometry of transformations of the complex plane. It has its origins in the discovery that the transformations obtained from complex analytical functions are conformal, that is, do not change the angles between curves. Let the unit disk be the set of all complex numbers $z = x + iy$ with $x^2 + y^2 < 1$, and let $f(z)$ be an analytical function. Then f can be thought of as a way of deforming the disk, moving each z to its image $f(z)$. What shape can this image be? According to the

Riemann mapping theorem, anything! (More accurately, anything that is topologically a disk; that is, a simply connected open set.)

The image of the disk may overlap itself (and the Riemann mapping theorem must be stated with due care in that case), but there is an important class of functions f for which it does not. Such an f is said to be univalent on the unit disk. At this point the hint of a connection between topological and metric properties of f arises. The larger the image of the disk under f , the larger, in some sense, f is. The largest univalent function should transform the disk into the largest possible image, namely the entire complex plane. (Actually, for technical reasons, it is necessary to slit the plane along a line segment.) Does this statement make any kind of sense, and if so, what is this largest univalent function? (It looks as if it ought to be interesting in the sense that extreme cases always are.) An attractive guess is the Koebe function, $z/(1-z)^2$, which has the required properties. This has the power series expansion $z + 2z^2 + 3z^3 + \dots + nz^n + \dots$ and it occurred to Bierberbach that its extremality might be reflected in its power series. If so, the n th coefficient in the power series of a univalent function should be no larger than n . In other words, metric extremality should follow from geometrical extremality. It is an appealing, natural and simple idea; the sort of thing that ought to be possible to decide one way or the other without much trouble — but, in fact, a proof turned out to be decidedly elusive.

More precisely, consider a complex power series of the (normalized) form $f(z) = z + a_2z^2 + a_3z^3 + \dots$ which is univalent in the unit disk. The Bierberbach conjecture is that $|a_n| \leq n$ with equality holding only for a rotation of the Koebe function $f(z) = z/(1-\omega z)^2$ where ω is a constant of

absolute value 1. (The circular symmetry of the disk forces us to admit rotations as well as the Koebe function itself.) Until last year the conjecture was known to be true only for $n \leq 6$; this was proved by Bierberbach himself for a_2 , by Löwner for a_3 , by P. R. Garabedian and M. Schiffer in 1955 for a_4 , by R. N. Pederson and M. Ozawa in 1968 for a_6 and by Pederson and Schiffer in 1972 for a_5 .

De Branges's proof is obtained by way of two related conjectures. The first, due to M. S. Robertson in 1936, refers to 'odd' power series $b_1z + b_2z^3 + b_3z^5 + \dots$ univalent in the unit disk, and asserts the inequality $|b_1|^2 + |b_3|^2 + \dots \leq n|b_1|^2$. A simple argument shows that the Robertson conjecture implies the Bierberbach conjecture. In 1967 N. A. Lebedev and I. M. Milin proved an inequality about logarithmic coefficients of power series, which in 1971 led Milin to conjecture a further inequality which would imply the truth of the Robertson conjecture. It is this Milin conjecture that de Branges proves: the Robertson and Bierberbach conjectures follow immediately.

The method used by Löwner in his successful attack on a_5 is to introduce a partial differential equation whose solutions approximate any function univalent on the unit disk. This differential equation represents an expanding flow, and it is possible to push information along the flow — for example, estimates of the size of Taylor series coefficients. De Branges's proof introduces auxiliary functions τ_k to hold the desired information, each defined by a differential equation involving all previous $\tau_1, \dots, \tau_{k-1}$. If these functions can be shown to have certain useful properties, then the Milin inequality follows. However, the combinatorics of the sequence of τ s rapidly becomes very hard to manage. Walter Gautschi verified by computer that the desired properties hold for all values of n up to 25 (implying the truth of the Bierberbach conjecture for $n \leq 26$), and later noticed that the general result followed from a theorem of Askey and Gasper about Jacobi polynomials.

De Branges originally obtained his result as the climax of a 385-page typescript of the second edition of his book *Square Summable Power Series*, where it appears as a corollary to a general theory making heavy use of functional analysis. His announcement of a proof was greeted with some scepticism. De Branges had earlier claimed a proof of the Bierberbach conjecture that turned out to be fallacious, and this may have damaged his credibility.

The conjecture has a history of leading mathematicians astray. As Carl FitzGerald says in a survey (*Not. Am. Math. Soc.* 32, 2; 1985) of the conjecture and the circumstances surrounding its solution: "The Bierberbach conjecture is not difficult; I have proved it dozens of times". Acceptance came only after de Branges visited the Steklov Institute in 1984, when his proof was confirmed by the members

of the Leningrad Seminar in geometric function theory. E. G. Emel'ianov found a simplification avoiding the use of functional analysis which was examined by mathematicians in the West, who made some further simplifications and agreed that de Branges really had solved the problem. The proof has now been made very short and direct: one version takes only five pages (FitzGerald, C. and Pommerenko, Ch. *Trans. Am. Math. Soc.*, in the press). De Branges independently found a similar simplification.

So now we know that the Bierberbach conjecture has a short and simple proof, based on standard techniques in geometric function theory. Something very close to the successful assault must have been attempted by dozens, if not hundreds, of mathematicians. Why was the proof not found earlier? A likely reason is the subtlety of the problem. The precise approach that was successful is very delicate, and without a great deal of previous work, partial solutions and 'experimental' results, it is unlikely that anyone would stumble upon the particular combination of conditions needed to make the proof work. Another possibility is that most of the attempts were somewhat half-hearted, because nobody seriously thought that the standard approach would work. Mathematicians habitually talk of certain methods as being "worked out", in the mining sense that everything of value that can be extracted from them already has been. It is usually considered to be a waste of time to attack difficult problems by 'exhausted' methods. Yet, here it has suc-

ceeded. There are other mathematical cases. Dehn's lemma in topology and the complex version of the Hahn-Banach theorem baffled everybody for years but were eventually solved by methods that are so natural that the real puzzle is why the solutions had not been spotted immediately.

The answer may be related to the structure of mathematical proofs. As an analogy, consider a game of chess. Chess openings have been analysed by generations of Grand Masters in the hope of finding a novel line of attack and springing a trap on an opponent who finds himself in unfamiliar territory and under pressure of time. But there are so many possible variations in chess that a complete analysis of even up to a dozen or so moves is not feasible — there is still room for an original line of development only a little off the beaten track. Nimzovich caused his opponents many headaches by opening with a knight, a move so ridiculous that nobody had bothered to take it seriously. The 'opening moves' in a mathematical proof are far more varied than in chess. A line of argument may seem to be a poor opening simply because everybody who tried to play the game in that way followed a similar pattern of obvious but poor moves, missing a brilliant variation that would crack the game wide open. Certainly it is unwise to write off a plausible line of attack just because nobody has yet been able to make it work. □

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Olfactory transduction

Welcome whiff of biochemistry

from Gordon M. Shepherd

As a detector of molecules, the olfactory receptor neurone is impressive, combining high sensitivity with a broad and rapid response. Given these properties, one might assume that the mechanism whereby these neurones transduce odour molecules into nerve signals would have been the subject of intense investigation. On the contrary, neuroscientists have gravitated towards the more tractable receptor mechanisms, such as those at the neuromuscular junction and in photoreceptors, where the stimulus is well defined and the receptors more accessible. Happily, the situation is beginning to change, and the report from Doron Lancet's laboratory on page 255 of this issue¹, of the apparent involvement of an odour-sensitive adenylyl cyclase in the sensory response, is an important step forward in the biochemistry of olfactory transduction.

The experiments were carried out in the olfactory epithelium of the frog (see Fig. 1). The initial step in odour transduction is believed to take place in the olfactory

cilia. Lancet's group obtained a cilia-enriched cell-free membrane preparation by dissecting out the simple nasal sacs of frogs, exposing them to a brief calcium shock, and differentially centrifuging out a preparation containing over 80 per cent cilia membranes. The basal level of adenylyl cyclase activity in this preparation was almost 15 times higher than in either the whole epithelium or whole brain, where activity is also high. The adenylyl cyclase activity was enhanced by GTP and compounds such as forskolin, and inhibited by guanosine 5'-O-(2-thio) diphosphate (GDP β S), thus meeting some of the common criteria for the activation of the adenylyl cyclase by a guanine nucleotide binding protein (G-protein). And ribosylation catalyzed by cholera toxin permitted the identification of a polypeptide with relative molecular mass (M_r) of 42,000, suggesting a stimulatory G-protein (G_s) within the general class of GTP-binding proteins (also called N proteins) activated by hormones and neuro-

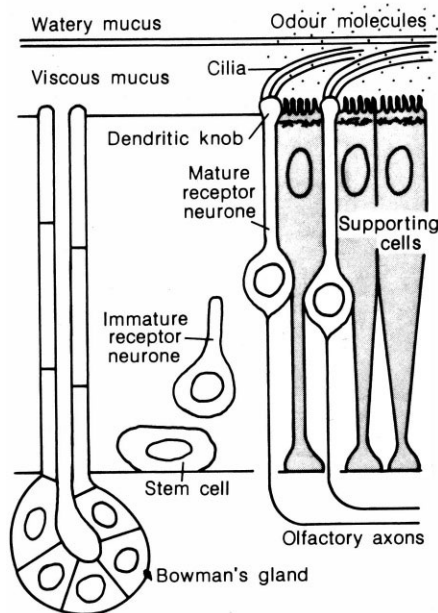


Fig. 1. Schematic diagram of the vertebrate olfactory epithelium. Mature receptor neurones arise during adult life by slow turnover of stem cells. Odour molecules are believed to act on receptor molecules (proteins?) in the membranes of the cilia and dendritic knob. Supporting cells have apical microvilli and are interconnected by a terminal web, as in other mucous membranes, thus limiting diffusion of absorbed molecules. They have glial-like properties and are believed to secrete the viscous mucus that bathes the surface. The thin watery mucus is thought to come from Bowman's glands.

transmitters.

If a G_s protein-adenylate cyclase system is involved in olfactory transduction, it should be affected by the presence of odour molecules. When the cilia preparation was exposed to a cocktail of four well-known odorant compounds, there was a GTP-dependent enhancement of the adenylate cyclase activity in a dose-dependent manner, at odour concentrations within the range of those used to stimulate receptor neurones in electrophysiological experiments.

From previous studies, it is known that field potential responses of the whole olfactory epithelium can be modulated by cyclic AMP analogues and phosphodiesterase inhibitors^{2,3}. The whole epithelium is a complex tissue (Fig. 1) in which the glia-like supporting cells and the glandular structures play undoubtedly important, but as yet undefined, roles. Because of these uncertainties and the limited data, the presence of a cyclic nucleotide cascade in the receptor neurones has been considered to be an open question⁴. The new study reduces the possibility of contamination by the other elements, and provides the clearest evidence yet of enzymatic processes related specifically to the sensory response.

The new data also fit nicely into an emerging outline for the sequence of steps that may be involved in olfactory transduction (Fig. 2a). Using this type of cilia-enriched preparation, Lancet's group has

previously reported the isolation of a 95K M_r glycoprotein that seems to be specific to the olfactory cilia⁵. They speculate that, like antibodies, this glycoprotein may have a constant and a variable region(s), the latter providing the diversity of sites required to account for the response of olfactory receptor neurones to a wide range of odour molecules⁶. Studies of other proteins that may be receptors are in progress elsewhere⁷.

By analogy with other second messenger systems, it seems a reasonable working hypothesis that binding of an olfactory molecule(s) induces an allosteric change in the receptor protein that causes activation of the G_s protein-adenylate cyclase cascade. One obvious advantage of this mechanism would be that the amplification built in to such an enzyme cascade would enhance the sensitivity of the receptor neurone; it could help to account for the ability of an individual neurone to be activated by single, or very few, molecules. Several types of receptor proteins might feed into the common enzyme pathway. Unfortunately, virtually nothing is known about the coupling of the adenylate cyclase to the presumed ionic channel(s) responsible for the receptor potential. This generates the impulse discharge that encodes the sensory response and transmits it to the brain.

The present report should provide a vigorous stimulus for further studies. Further biochemical characterization of the putative receptor protein and the G_s protein-adenylate cyclase pathway is urgently needed. Cloning of the genes for, and sequencing of, the olfactory receptor and G_s protein would then be not far from reach; the cloning of the gene for the α -subunit of transducin, the G protein in photoreceptors, has recently been reported⁸. Electrophysiological techniques have improved to the point where intracellular recordings can be routinely obtained from the olfactory receptor neurones, in spite of their very small size. Two possibilities deserve testing: first that the common action of cAMP causes phosphorylation of a channel protein and opening of ionic channels, resulting in the depolarizing receptor potential observed in response to odour stimulation; second, that there is a more direct effect on membrane conductance, similar to the direct action of cyclic GMP on membrane channels in rod photoreceptors^{9,10} (Fig. 2b). Whatever the mechanism, the extremely high sensitivity of olfactory neurones to injected currents suggests that small conductance increases, involving few channels, could produce large receptor potentials¹¹. Odour activation of single channels, and their control by adenylate cyclase, can be studied in patch-clamp recordings from cultured olfactory neurones¹² or in reconstituted membranes¹³.

Interest thus quickens. Perhaps there are other transducer mechanisms and pathways, such as through polyphos-

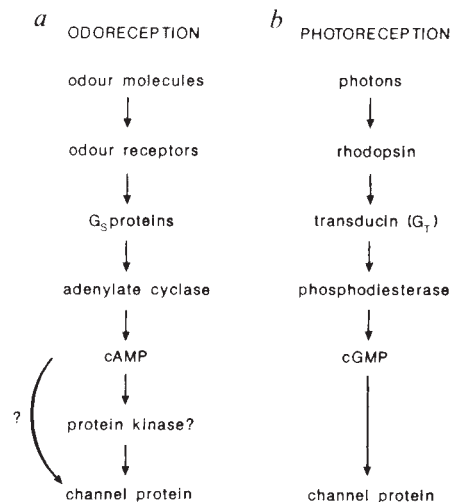


Fig. 2. a. Summary of proposed enzymatic cascade involved in olfactory reception⁶. b. The enzymatic cascade involved in visual reception^{9,14} for comparison.

phoinositol breakdown. The receptors could be modulated by neurotransmitters from autonomic fibres and nerve terminals, as well as by hormones carried through the rich vasculature of the epithelium. Perhaps the mechanisms of olfactory reception will turn out to be similar to those of the immune system, as noted above. There are intriguing similarities; for instance, as well as recognizing a variety of molecules, olfactory neurones are continuously generated throughout adult life from precursor cells (Fig. 1). If the analogy with the immune system finds support, we may eventually characterize olfactory transduction as involving 'odogens' which stimulate 'odobodies', leading to changes in activity of the intracellular enzymatic pathways discussed above and, perhaps, also to the release of extracellular signals that communicate with the surrounding supporting cells and regulate the differentiation of new receptor neurones from the precursor cells. Undoubtedly, there are pleasant surprises awaiting us right under our noses. □

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Optics

Reflections on closed loops

from David K. Lynch

REFLECTION and refraction at the surface of gently rippled water produces images containing a variety of closed curves, the shapes of which depend on the spatial, or angular, distribution of the incident light field. The loops usually represent the distorted boundaries of high contrast features in the scene. In these columns¹ recently Thomas Gold discussed the formation of closed loops in reflections of linear objects such as the mast of a sailing boat, but point sources and objects extended in two dimensions also form closed-loop images. Here, I compare the loops resulting from reflection and diffraction of all three types of source.

All real sources are extended to some degree but sources that approximate to a point, such as the Sun and Moon, create a myriad of rapidly formed loops (Fig. 1), usually in the shape of distorted ovals^{2,3}. Because the water contains many surfaces inclined at precisely the correct angle to reflect light to the observer, the casual observer sees the loops — which form almost too rapidly to follow — coalesce to create the familiar glitter patch^{4,5}. These are readily seen in any body of water illu-

minated by a point source high overhead, such as bath tubs, swimming pools and coffee cups. The statistics of such reflections have been discussed by Longuet-Higgins⁶, but the details of loop formation have not been well studied.

Theoretical studies of point sources show that there is not a one-to-one correspondence between the source and its image: that is, the solutions are not unique. For example, when the local radius of curvature of the water is twice the distance between the observer and the surface, the image of a point source is spread into a two-dimensional patch of light.

For linear sources of light such as masts, the reflected structure depends on the angle of the line of sight and the orientation of the source^{1,3}. Since a vertical mast separates two extended, nearly identical featureless regions, the blue sky seen inside a loop comes from one side of the mast and the blue region surrounding the loop comes from the other, a distinction that cannot be made for point sources.

With extended sources, such as mountains and clouds, the closed curves form landpools and skypools (W.C. Livingston,

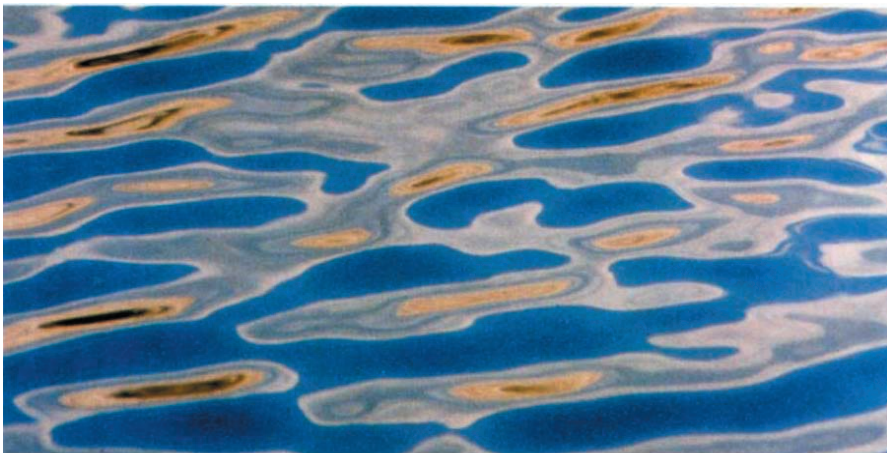
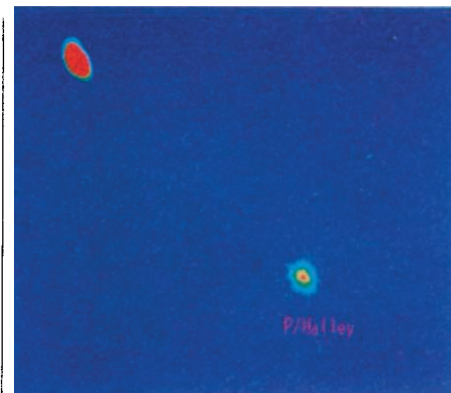


Fig.2 (top) Skypools and landpools in a reflection of an extended source.

Fig.4 (bottom) Refracted images of the sandy bottom of a lake.



First sight of Halley

CCD image of comet P/Halley (1982i) obtained on the 18 April 1985 with the 4.5-m Multiple Mirror Telescope of the Fred L. Whipple Observatory (MMTO). The integration time was 10 minutes and the seeing FWHM was 0.8 arc seconds. The comet image extends 5 arc seconds in the sunward direction with a brightness V of 18.7. The object at the top left is a binary star whose images have been smeared in following the comet's motion. The contoured bands of the cometary image indicate that the coma has been resolved. The image was digitally processed by K. Hege of MMTO. The first spectra from the coma are discussed on page 241.

personal communication), regions of reflected blue sky light surrounded by darker regions from the landscape and vice-versa (Fig.2). Except for the distortion, landscape re-imaging is often extremely accurate on water in terms of surface detail. The reflected blue sky seems almost white at the boundary of the skypool. This is simply the reflection of the true brightness and colour distribution of the sky made readily visible because many tens of degrees of vertical sky are compressed into a small angle. However, skypools are not distorted images of the entire sky because only a small part of the sky is actually re-imaged; the upper and lower parts of the skypool originate from the same part of the horizon and approximate to mirror images.

Refraction can also render objects of all three categories into closed curves. The most common examples can be seen on the bottom of a swimming pool. High contrast point-like sources (most commonly the Sun) appear as loops, analogous to those formed by reflected points. Such linear sources, distorted by refraction, become the familiar bright webs on the bottom when light is focused by the wavy surface of the water⁷ (Fig.3). Because of dispersion, these loops also show brilliant colours. Extended sources, such as a bright rocky or sandy bottom, also display closed contours when viewed at low incidence. In these images the phenomenon is developed to its most fascinating complexity (Fig.4).

Much of the structure in both reflected and refracted distortions depends on the

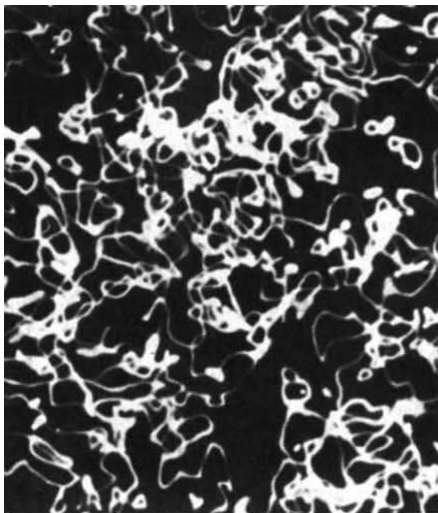
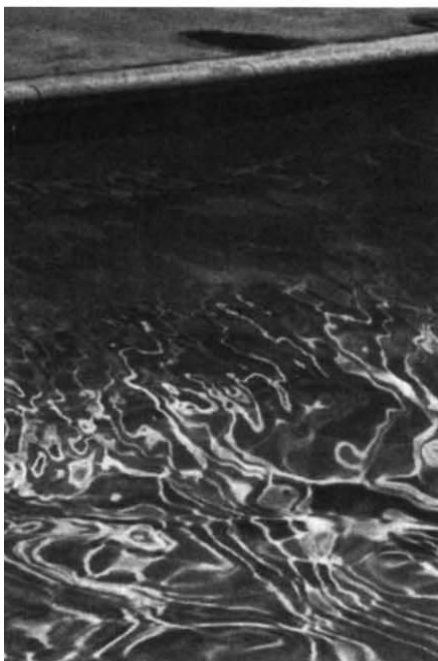


Fig.1 Reflection of a point source: the moon.

Fig.3 Refracted image of a linear source on the bottom of a swimming pool.



duration of the observation and the spatial distribution of the source. If photographs of point sources reimaged by the water are taken with shutter speeds far shorter than the period of the waves, the image usually shows many sharp loops, together with partial loops caught as the shutter opens or closes in the middle of a trajectory. Exposures much longer than the wave period result in overlapping images. For a point source against a dark background, overlaps are simple but photographs of an extended source create patterns of colours and shapes that rival the finest impressionist art. □

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Geomagnetic field

Volcanic record of reversal

from Jean-Pierre Valet, Carlo Laj and Piotr Tucholka

ON PAGE 230 of this issue Michel Prévot and his colleagues report the most detailed palaeomagnetic record of a reversal in the Earth's geomagnetic field to have been obtained from lava sequences¹. Because the type of rock in which the record is preserved determines certain characteristics of the record itself, it is of particular interest to compare some characteristics of the Steens Mountain record of Prévot *et al.* with similarly detailed records obtained from sediments.

Important data on the morphology of the transitional geomagnetic field have been obtained in the past decade from palaeomagnetic records of reversals. The first big step was the recognition that the transitional field is not dipolar². K.A. Hoffman then attempted to recognize an average field configuration³; in some cases there is an indication of axial symmetry⁴ but non-axisymmetrical components are present in most detailed results, both for single transitions⁵ and for sequences of reversals⁶. Although many attempts have been made to determine the harmonic content of the transitional field^{7,8}, further clarification of this problem needs records of transitions from the Southern Hemisphere.

The records have generally been obtained from sediments or from lava sequences. Magnetization of sediments is associated with the continuous process of sedimentation so that the resulting signal depends on the deposition rate and on the time necessary for the locking in of the remanence. If the acquisition process is very prolonged, the result can be a heavy smoothing of the signal. Laboratory studies^{9,10} have shown that magnetization is locked in at a depth of about 10–20 cm in the sediment, a value which has recently been confirmed by a novel method¹¹. Moreover, the pattern of the record itself can be used to determine the importance of this effect. Despite these apparent drawbacks, it is only sediments that can provide continuous, multiple records of the same transition from widely separated localities as well as records of sequences of reversals.

The magnetization in lavas is acquired in a very short time while the lavas are cooling down in the ambient field. Therefore, a sequence of lava flows provides a succession of instantaneous pictures of the transitional field and so is very sensitive to the rapid directional changes of the field on a very short timescale. Determinations of the absolute palaeofield intensity from lava, unlike sediments, are particularly reliable but, unfortunately, although the sequence of flows can be well established, it is impossible to determine the exact tim-

ing of the eruptions. Also, records from lavas provide neither verification of results from multiple profiles nor sequences of records from consecutive reversals.

The transition recorded at Steens Mountain in Oregon is characterized by the succession of two distinct phases, the first being a complete flip from normal to reverse of the vector, while the second is a normal–transitional–normal rebound. Most of Prévot *et al.*'s discussion is given over to the field variations associated with the second phase. During this period, the directional changes are mostly scattered far away from the meridional plane and the virtual geomagnetic poles describe large longitudinal variations. There are also two important directional jumps, which the authors interpret as geomagnetic impulses associated with global changes of the field with time constants of 1–2 years. Note that these impulses occur while the field configuration is away from axial symmetry. Following the suggestion of the authors that such impulses reflect an increase of the turbulence within the Earth's core, the results also suggest that the whole field configuration is affected by this increase. The authors note that time constants of the same order of magnitude as the impulses have been observed for the 1969 secular variation jerk. It is thus important to connect this observation with a recent theoretical prediction by J.L. Le Mouél¹² that some of the observed features of the secular variations should persist during a transition.

The two phases of the transition are separated by an episode of normal directions and pretransitional intensity values, which is interpreted by Prévot *et al.* as the temporary re-establishment of the dipole field configuration. In view of the thickness of the recording zone, they argue that the first episode lasted half the time of the second, so that the whole record would correspond to a single reversal. But this argument rests on the assumption that the volcanic activity was regular and uniform which is, at least, questionable.

Alternatively, therefore, it can be suggested that the polarity change was completed at the end of the first phase and that the second phase is a record of an excursion that is quite separate from the major reversal. Of course, this interpretation implies very different characteristics of the transitional field, closer to those that are commonly observed in sediments. In particular, the directional changes are much more regular and the resulting directional path is highly confined in a narrow longitudinal band, suggesting a degree of organization of the transitional field. We note also that the first fast

impulse lies well within the meridional plane. Fast changes within transition paths are also apparent in sedimentary records but are often disregarded as possible disturbances of sedimentary or other origin. Perhaps in the light of the Steens Mountain results they will merit new consideration.

If the second episode reflects an excursion of the geomagnetic field that is unrelated to the polarity reversal, the Steens Mountain record provides evidence that the field behaviour associated with these two phenomena is different. The core processes during reversals and excursions would, at least in this case, not be intimately related. In particular, the hypothesis of K.A. Hoffman¹⁴ that palaeomagnetic excursions are aborted reversals would not be valid.

Although Prévot *et al.* certainly have written an important chapter, the history of palaeomagnetic records of transitions is not finished. Our knowledge of the transition field should be increased by multiple and detailed records of the same reversals. Thus, records from sedimentary sequences offer the most promising per-

spectives. In particular, very important results can be expected from records with very high sedimentation rates. This should allow time resolution in a range similar to that which is possible for volcanic rocks and so should lead to a reconciliation of the sedimentary and volcanic records. □

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Climate modelling

The climate as natural oscillator

from Gerald R. North

THE Earth's climate exhibits great variability at timescales centred at 100, 41 and 20 kyr, as shown convincingly by J. Hays *et al.* (*Science* **134**, 1121; 1976) using data from marine sediments. As these oscillations happen to be the dominant periods of the variations in the Earth's orbital elements (eccentricity, obliquity and perihelion at summer solstice), they have stimulated a revival of the astronomical theory of the Quaternary glaciations (see Milankovitch and Climate eds Berger, A. *et al.* Reidel, Dordrecht, 1984).

Steady-state atmospheric climate models show remarkably little sensitivity to orbital changes, leading to a major crisis in climate theory over the past decade. An interesting line of reasoning has been pursued by Barry Saltzman and colleagues (for example, *J. atmos. Sci.* **41**, 3380; 1984). To avoid the issue of sensitivity, Saltzman suggests that the system is a natural nonlinear oscillator with a period of about 100 kyr. The tiny orbital forcing merely acts as a pacemaker providing a phase and precise frequency to lock with.

Although it is premature to call the Saltzman theory conclusive, its elegance and generality make it essential reading for ice-age buffs. The model is highly idealized, keeping track of only a few important dependent variables. It consists of a set of first order (in time) differential equations — the prognostic equations — for the perturbed variables: land-ice volume, sea-ice volume and deep ocean

temperature. The differential equations are coupled through simple polynomial forms up to the third degree in powers of the variables. The solution for equilibrium (all variables vanishing) exists, but it is unstable to infinitesimal perturbations from equilibrium for reasonable values of the coefficients in the system. The free solution state is quickly drawn away from the origin to a limit cycle with a period of about 100 kyr.

The dominant physical process in the model is the sea ice cover. When it is large, the planet has a large albedo leading to an overall cooling and further growth of ice volume by expansion of the freezing line towards the equator. On the other hand, large areas of ice-covered ocean inhibit evaporation and therefore starve the glaciers. Other feedbacks, such as inhibition of heat transport towards the pole under the sea ice and the subsequent buildup of deep ocean temperature, act in the same general manner. If the time constants for these processes are different enough they can lead to oscillations with rather asymmetrical growth and decay time characteristics. The longest time constant introduced in the problem as a parameter is about 7 kyr in the thermal inertia of the deep ocean. The nonlinear dynamics of the system translates this relatively short timescale into the much longer natural oscillation period. The sharp termination of ice volume in the data is one of the unanswered questions about the 100-kyr

cycles. This sawtooth time series, consisting of slow ice growth and abrupt terminations, seems to be a natural property of the Saltzman model, arising from the differences in time characteristics of the individual components. Parameter sensitivity studies show that the essential character of the oscillation is unchanged by small departures from the nominal values.

Although the Saltzman model is attractive because of its logical consistency and systematic formulation, it is not alone in the arena of contenders for ice-age glory. M. Ghil and colleagues (*J. geophys. Res.* **88**, 5167; 1983) suggest an oscillator model based on a short natural period (about 10 kyr). When driven by the Milankovitch frequencies, the solutions exhibit combination tones, one of which is near the 100-kyr period. In their theory, R. Peltier and J. Hyde (*J. atmos. Sci.*, in the press) emphasize feedbacks associated with the bedrock response. The latter theory does not exhibit free undamped oscillations, but it does seem to have a strong response at the 100-kyr period when driven by a forcing frequency near a harmonic of this natural period, say 20 kyr.

There has never been a shortage of ice-age theories, because many of the component processes of the system cannot yet be modelled. The result is a group of schemes replete with free parameters and fudge factors — the Saltzman theory has about 10 such factors and others are comparably endowed. The biggest challenge facing the ice-age system theorist seems to be finding a risk that a model can run in the face of hard evidence as opposed to fitting the lone time series of ice volume. In terms of fitting the ice volume time series it is hard to surpass the phenomenological model of J. Imbrie and J. Z. Imbrie (*Science* **207**, 943; 1980), purely because of its simplicity. The model included one time constant for ice volume growth, one for its decay and a scale parameter for the forcing strength. The result was a rather good fit to the sawtooth time series. The main task now faced by modellers is to construct theories that derive these parameter values from more fundamental principles.

The Achilles heel of simplified theories modelling time behaviour of ice volume is their sensitivity to the parameterization of snowfall over the ice sheets. This is especially grim as the most detailed general circulation models of the atmosphere seem unable to compute the precipitation rates adequately, particularly near the poles — just where it counts in an ice-age theory. The next decade may lead to a better understanding of such precipitation, producing a breakthrough comparable to geological evidence in terms of eliminating classes of ice-age theories. Meanwhile, modellers can explore the vastness of parameter space unconstrained by cruel realities. □

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Simpler DNA sequence representations

SIR—The method of DNA visualization presented by Hamori^{1,2} admits of a simpler representation which may be more generally useful. In Hamori's technique, each of the four nucleotides is represented by a vector in three-dimensional space having a characteristic, but variable, orientation. DNA structure then may be viewed from any chosen perspective in two-dimensional plots on computer graphical monitors. Different aspects of structure are examined by varying the geometric relationships among the four elemental vectors.

I suggest that a particular, canonical choice of vectors has certain advantages, both for exploring structure and for comparing sequences. Figure 1 illustrates an intronic sequence based on this approach, wherein successive guanine (G) residues are represented by unit vectors in the positive x-axis direction, complementary cytosine (C) residues correspond to negative x-axis unit vectors, and thymine (T) and adenine (A) residues correspond to unit vectors in the positive and negative y-axis directions respectively.

With this choice, all sequences can be represented in two dimensions in a unique manner, and differences in sequence structure become immediately apparent. In Fig. 2, which compares two actin genes, the regions of divergence of the exonic sequences are clear.

Furthermore, this method of embedding DNA sequences in a two-dimensional metric space permits the consistent application of several standard concepts based on distance measures. Two are worth mentioning. Plots of cumulative Manhattan distances from a site of origin (for example, the initiation codon) against position number can be useful in pinpoint-

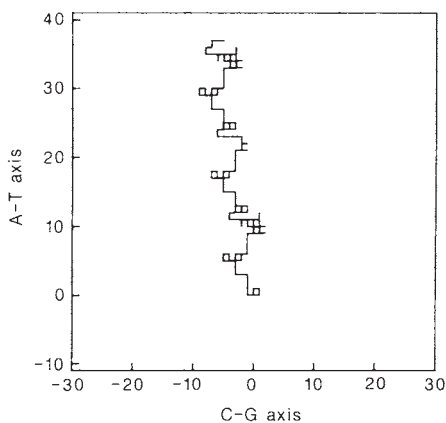


Fig. 1 Graphical representation of the 186-base pair (bp) nucleotide sequence of human BK papovavirus BK enhancer³, showing three 12-bp repeats. The sequence begins at the bottom, at coordinate position [0,0], and reads (5' to 3') towards the top. Successive G, C, T and A nucleotides are depicted as coordinate shifts of one unit in the +x, -x, +y and -y directions, respectively.

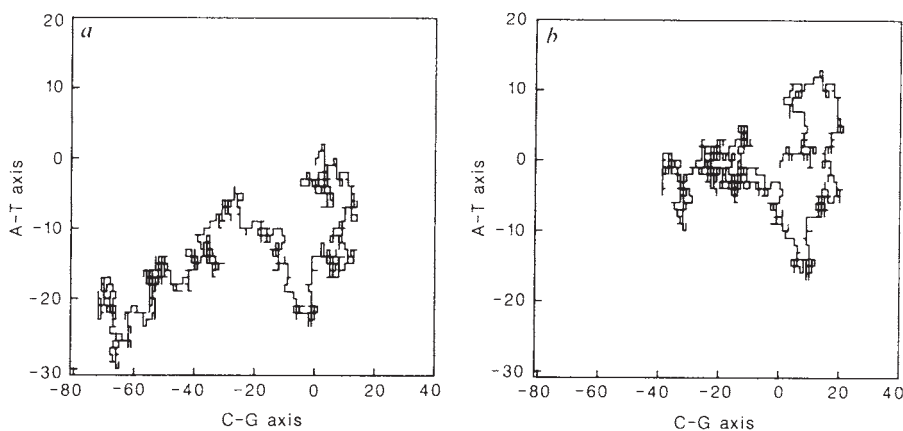


Fig. 2 Nucleotide sequence representations of two actin genes from *Drosophila melanogaster* (Canton S strain). The single intron has been removed from each, leaving a 1,131-bp exonic sequence. Data were obtained from the GenBank library. *a*, Cytological locus 79B; *b*, cytological locus 88F.

ing similarities in structure of sequences coding for the same type of product; this ideally should be done after alignment for maximum sequence homology.

Finally, the general properties of classes of sequences (for example, actin genes from different groups of organisms, different types of immunoreceptors) can be examined by calculating the fractal dimension, in this space, of each sequence. This is simply the ratio of the logarithm of the total Manhattan path length to the logarithm of the distance of the end-

point of the sequence from its origin: $\log [n(A) + n(G) + n(T) + n(C)] / \log [n(G) / n(C) + |n(T) - n(A)|]$, where $n(X)$ is the number of occurrences of nucleotide X in the sequence.

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Similarity of vaccinia 28K, v-erb-B and EGF receptors

SIR—Vaccinia virus, a member of the Poxviridae, has a large, double-stranded DNA (about 187,000 base pairs) and replicates in the host-cell cytoplasm. The genome contains more than 100 genes, some of which probably code for the enzymes involved in the viral transcription process. Recently, similarities were found between the sequences of the vaccinia 19K protein, EGF, transforming growth factor type 1, and several other mammalian proteins^{1,3}.

Using the FASTP rapid similarity search⁴ and other programs of the Protein Identification Resource system, we have now found unusual sequence similarity (Figs 1,2) between the vaccinia virus 28K proteins a major late protein of unknown function, synthesized after DNA replication and the carboxyl ends of the v-erb-B transforming protein of avian erythroblastosis virus⁶ and, to a lesser extent, the related human epidermal growth factor (EGF) receptor precursor⁷.

When we used the ALIGN program to evaluate these similarities, the 28K protein and a portion (345-584) of the carboxy-terminal domain of the v-erb-B protein produced an alignment score of 4.9 s.d. (mutation data scoring matrix with 6 added to each term, penalty of 6 for a break in either sequence, 300 random

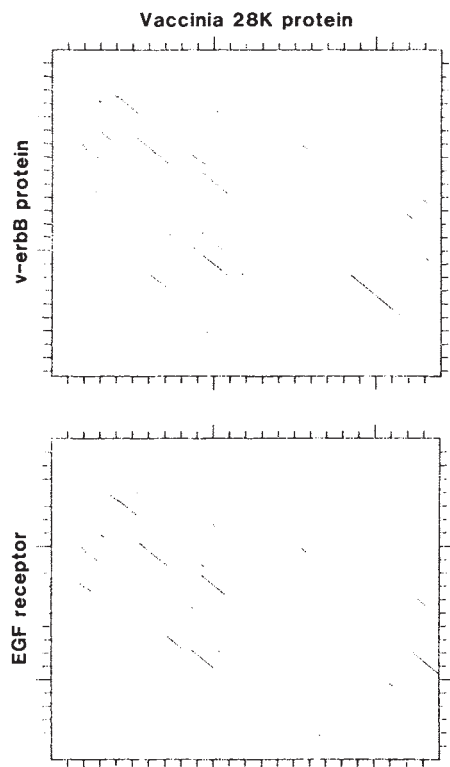


Fig. 1 Graphic matrix plots of the vaccinia 28K protein, the v-erb-B protein (350-604), and the EGF receptor precursor (920-1,170). Each point represents a score of 20 or more over a span of 25 amino acids using the mutation data scoring matrix.


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28K  M V I A G A K S K F P R S M L S I F N I V P R T M C K Y E L E L T H N E N I T G A M F T T M Y J I R N N
v-erbB M I D A D S R P K F - R E L T A E F S K M A R D P P R Y - L - V I Q G D E R M H L P S P I T D S K F Y R T
EGF r.  M I D A D S R P K F - R E L T I E F S K M A R D P Q R Y - L - V I Q G D E R M H L P S P I T D S H F Y R A

28K  L G L G D D K L T I E A T E N Y F L D P H N E V M P L I N N T D M T A V I P K K S G R R K N K N N V I
v-erbB L M E E E D I M E D I V D A D E Y - L V P H Q G G F N - - S P S T S R I T P L L S S L I A T S N H S A T N C
EGF r.  L M D E E D I M D D V V D A D E Y - L I P Q Q G F F S - - S P S T S R T P L L S S L S J A T S N H S T V A C

28K  F R Q G S S P L C I F Q T R K K I N I Y K E N M E S A S T E Y T P T G D N K A L I S K Y A G I R I L N
v-erbB I D R N G - Q G - H P V R E D S F V Q R Y S S D P T G A L T E - E S I D D G F L P A P E Y V - - N Q L M
EGF r.  I D R N G L Q S - C P I K E D S F L Q R Y S S D P T G A L T E - D S I D D T F L P V P E Y I - - H Q S V

28K  V Y S P S T S I R L N A I Y G F T N K N K L E - - K L S T N K E L S Y S S P L Q E P I R L N
v-erbB P K K P S T A M V Q N Q I Y - - N F I S L T A I S K L P M D S R Y Q N S H S T A V D N P E Y L N
EGF r.  P K R P A G S - V Q N P V Y - - H N - Q P L N P A - - S R D P H Y Q D P H S T A V G N P E Y L N

```

Fig. 2. Alignment of vaccinia 28K protein (28–229), v-erb-B protein (372–562), and EGF receptor precursor (952–1,140). Identities between the 28K protein and either of the other two are boxed.

permutations). A score of 7.2 s.d. was obtained if portions of the 28K protein (28–229) and the v-erb-B (372–562) were compared. Comparison with a portion (952–1,140) of the cytoplasmic domain of the precursor EGF receptor produced a score of 5.6 s.d. Because of the exhaustive nature of the FASTP search, values in this range indicate a very interesting similarity that is likely but not certain to have biological significance⁴.

Such similarity can result from common ancestry or from convergent evolution of proteins with similar structures or functions. For example, it may be advantageous for the virus to mimic certain host proteins. It remains to be seen if more vaccinia proteins have sequences similar to host proteins. Perhaps the relationship between vaccinia virus and its host cell will become clearer when the entire genome of this virus is completely sequenced and more host sequences are available.

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Did Burton synthesize diamonds in 1905?

SIR—In a letter to *Nature* of 24 August 1905¹, C. V. Burton, a prominent physicist, claimed to have synthesized diamond at ambient pressure by an unusual method. It may seem odd that the first response to this communication should appear some 80 years later, but the strange fact is that, for some inexplicable reason, this disclosure was lost to the literature.

Although a brief abstract appeared in *Science Abstracts*, it was not abstracted by any of the three chemical abstracting journals available at that time nor has any chemical treatise concerning carbon referred to it. Despite the voluminous literature relating to synthetic diamonds, not one author has referred to Burton's work. By chance, one of us (F. S.), found a reference to Burton's letter in a book on gem

stones by W. Goodchild², published in 1908 when the memory of Burton's disclosure was still fresh in the author's mind. Burton gave only a skeleton outline of his procedure and promised a further communication. However, a meticulous search has not disclosed such a communication.

Although Burton claimed to make diamond in the region of temperature and pressure in which graphite was the stable form of carbon, there is no reason why he should not have been successful in obtaining diamond in a metastable state. His method was to dissolve carbon in a lead-calcium alloy in which carbon is more soluble than in lead alone, then supersaturate the lead by oxidizing the calcium with steam at a "dull red heat" thus removing it from the alloy. The carbon precipitated in the form of diamond. Ostwald's law of successive reactions, also now lost from physical chemistry textbooks, provides for a metastable phase to appear before the stable phase, and at 500°C, the conversion of diamond to graphite is exceedingly slow. The data Burton gave on the crystals he obtained, their octahedral form, the refractive index, and the resistance to chemical attack are consistent with his claim to have made microscopic diamonds.

We have attempted to repeat Burton's work. In the absence of any experimental details, it is difficult to be sure that we followed his procedure exactly, and we had some difficulty incorporating the carbon in the alloy but we successfully solved that. We decomposed the alloy with steam at 550°C, our estimate of a dull red heat. The grey crust was treated with acid and washed well to remove all lead and calcium salts. We obtained a black powder in which were embedded many transparent crystals which scintillated with considerable fire in reflected light. They were at most a few micrometres in size. Burton claimed that he made only diamonds and no graphite. We obtained considerable amounts of black powder which may or may not have been graphite which made it impossible to separate the tiny crystals for precise identification. The crystals had a high refractive index and the powder scratched glass. X-ray powder diffraction showed a strong peak at 0.208 nm which is the strongest peak for diamond, but only weak for graphite. Strangely, the

strongest peak for graphite was absent, though two other peaks at 0.161 nm and 0.148 nm were still present. The strongest peak was an unidentified one at 0.251 nm. On the basis of this information, we cannot claim unequivocally that diamond was produced, but combined with the other properties, there is a strong presumption that diamonds were made and, therefore, Burton had synthesized diamonds in 1905, half a century before General Electric had made them in the stable region of high temperature and pressure. He deserves recognition in the Hall of Fame which includes Moissan and Hannay.

Thanks are expressed to the National Science Foundation for providing a grant to enable this work to be done, to L. W. Zelazny for his help in providing the X-ray data, and to F. D. Bloss for assistance with the refractive index.

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Misapprehension over Alzheimer's disease

SIR—Stanfield *et al.* make the *en passant* observation that degeneration of the neurones of the nucleus basalis of Meynert "are the likely cause of Alzheimer's disease"¹. This is not so. However, it is a widely held misapprehension. Other nuclei besides the nucleus basalis are similarly afflicted with tangles and show similar cell loss in Alzheimer's disease^{2,4}; lesions to the nucleus basalis area in animals produce few of the clinical and none of the pathological symptoms of Alzheimer's disease. The article commonly quoted⁵ as support for the cholinergic hypothesis has the beautifully ambiguous title "Alzheimer's disease: a disorder of cortical cholinergic innervation". This title is correct in only one of its senses.

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a rather technical character which are not provoked by articles or letters previously published (where Matters Arising remains appropriate).

An uneasy relationship

D. M. MacKay

Cross-Currents: Interactions Between Science and Faith.

By Colin A. Russell.

Inter-Varsity Press, Norton Street, Nottingham NG7 3HR, UK: 1985. Pp.272. £7.95.

To be published in the United States in August by W. Eerdman.

THE popular image of science has undergone some curious reversals of fortune over the past century. Fifty years ago, the rabble-rousers of the *avant garde* preached the gospel of "scientific humanism", according to which science was the ultimate answer to all the problems besetting mankind, including those traditionally the province of religion. In more recent years the fashionable trend has been to turn upon science as itself the origin, rather than the remedy, of the ills of our age.

In times like these the corrective of a broader historical view is much needed, and Colin Russell's book seems admirably designed to meet the need. As Professor of the History of Science and Technology at the Open University he has both the knowledge and the communicative skills to bring alive the ways in which religious and scientific ideas have interacted over the centuries to shape our conception of the natural world. Following in the footsteps of such pioneers as R. Hooykaas (whose classic *Religion and the Rise of Modern Science* is frequently cited), he shows how from early Greek times onward people's ideas of God, or the gods, have coloured their attitude to nature and helped or hindered their efforts to understand it. In the end, refuting popular ideas to the contrary, he is able to marshal "powerful historical evidence of a massive mutual debt between Christianity and science":

Conflict there has certainly been, but always for reasons that are peripheral to the real issues with which science and Christianity are concerned. Only in that limited, even localized, sense has there been anything like even an "uneasy truce". Desperate attempts to evacuate the Christian faith of its essential content, so as not to offend the susceptibilities of "scientific man", are not merely misguided but leave most scientists profoundly unimpressed [p. 252].

More negative historical phases are not neglected. The mixed reception given to Copernicanism is carefully analysed, and the popular notion that the heliocentric model was seen by contemporaries as a "demotion of man" is cogently questioned. Rhetoric's long-lost treatise designed to demonstrate Copernicus's orthodoxy, recently re-discovered by Hooykaas, takes a line rather similar to Kepler's in pleading that Holy Scripture (with its poetic references to the "fixity" of the Earth) was not intended to be used as a textbook of science. One gets the impres-

sion that if Galileo had been a little less aggressively tactless, the Roman Church might have been coaxed to recognize in time (rather than two centuries after the fiasco of his trial) the theological innocence of his proposals.

The part played by Puritanism in the rise of modern science has been much debated since R. K. Merton highlighted it. Russell gives a fair picture of the controversy, concluding that

underlying the bewildering variety of parties associated with science was a common core of biblical allegiance common to Puritanism, to the wider Calvinism and indeed to Protestantism as a whole. Of the resonance between that allegiance and the growth of science there can be no possible doubt [pp. 83-84].

Moreover

[People] are surprised that the tight-lipped, joyless exponents of an iron religious creed could ever unbend to consider such frivolities as scientific experiments, let alone have the wit to understand them. Yet that is only because "Puritan" and "puritanical" are given a totally unhistoric meaning today. The early Puritans, like Bunyan, did take their faith seriously, but they laughed and made music and love like anyone else, and they, more than their contemporaries, delighted in the world of nature [p. 82].

An illuminating section on the Darwinian controversy draws on recent scholarship, especially on the work of J. R. Moore, to reassess the parts played by religious (and anti-religious) convictions among both supporters and antagonists of evolutionary theory. Despite their hostility to the established Church, T. H. Huxley and his Victorian friends were curiously soft-centred in their attitude to nature — almost as if they hoped to find in "her" the God they had lost. Huxley's view that "living nature is not a mechanism but a poem" typifies the Romantic strain in much agnostic thought of the day. Russell gives some fascinating glimpses of the pseudo-religious fervour with which the new and optimistic "scientific" creed was followed. It would seem (though he does not directly say so) that the aggressively anti-religious X-club founded in 1864, together with such vocal protagonists of "Victorian scientific naturalism" as J. W. Draper and A. D. White, showed in their vehemence and their disregard for awkwardly relevant facts quite as much extremism as we nowadays deplore in anti-evolutionary fundamentalism. Conversely, Russell makes clear that a num-

ber of convinced Christians were from the outset enthusiastic (perhaps too uncritically so) in support of Darwin's biological theory (as distinct from the atheistic metaphysics that borrowed its name and prestige). The fact that men of the stature of Joule, Maxwell, Kelvin and Faraday remained quietly convinced believers is also cited against any idea that the leaders of Victorian scientific thought, as a body, felt themselves to be at war with religion.

The concept of science as a potential boon to man, of which Francis Bacon made so much, was evidently alive already in the thirteenth century. It is all the more curious that, despite the declared endorsement of it by the fledgling Royal Society, this idea for so long bore so little

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Victorian protagonists — T. H. Huxley (left) and Michael Faraday.

practical fruit in technology. Painstaking improvements in craftsmanship brought rich dividends. Advances in the design of chronometers, for example, revolutionized the art of navigation. But according to Russell it is not until the middle of the nineteenth century that we find scientific insight as such contributing significantly to technological advance; and even then, as the story of thermodynamics shows, the traffic was by no means all one-way.

Readers who wonder how some modern bishops' views on miracles fit into the historical picture will find Colin Russell well prepared to answer; and chapters on the impact of quantum theory on theology, and of the Christian doctrine of stewardship on ecological thinking, ensure that contemporary issues are not overlooked. Though Professor Russell writes as a Christian, he maintains a judicial posture and a scholarly regard for documentation that makes his book specially suitable for students and those concerned to see the record straight. Appetizing as well as authoritative, it should be required reading for teachers of science as well as of religion, whether in or out of the pulpit. □

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Cloning cookbook for the laboratory

Kevin Struhl

A Practical Guide to Molecular Cloning. By Bernard Perbal. Wiley: 1984. Pp.554. Hbk £68.85, \$59.50; pbk £39.90, \$34.50.

TEN years ago, molecular cloning was an art that was practised only in a few Californian laboratories. Now, with everyone and his brother involved in recombinant DNA manipulations, the field has exploded into a vast and complex technology that is far beyond the ability of an individual to learn and remember completely. Furthermore, because these techniques have become invaluable in almost all fields of biological science, it is frequently the case that those who want to use them are not trained as molecular biologists. In short, there is a great need for a cookbook, which contains a reasonably complete collection of recipes that are easy to follow and up to date.

Three years ago Cold Spring Harbor Laboratory published such a collection, *Molecular Cloning: A Laboratory Manual* by Maniatis, Fritsch and Sambrook. This book is omnipresent in molecular biology laboratories and is utilized to the point where it is frequently referred to as "The Bible". Although experts usually use shortened versions of the procedures, the methods are clearly described and they do work. Indeed, the only shortcoming of this manual is its age. It does not contain anything on the production of monoclonal antibodies and related methodologies, now standard tools of the trade, and the section on expression vectors for the controlled production of gene products is weak and out-dated. An updated version is clearly necessary, and the authors are, in fact, preparing a new edition.

What does *A Practical Guide to Molecular Cloning* offer that the earlier book lacks? Unfortunately, the answer is not much. The most practical addition is that of DNA sequencing using single-stranded DNA vectors and primer extension with di-deoxy nucleotide precursors. However, most commercial suppliers enclose an equally detailed protocol on purchase of the reagents. The author also describes a number of techniques for the purification

of large quantities of DNA. These methods are seldom used and the descriptions read suspiciously like the promotional literature from the companies that sell the products.

Given the need to include newer techniques, Perbal devotes an inordinate number of pages to unnecessary matters. More than 20 per cent of the book is spent on lists of restriction endonucleases and vectors; such information, although useful, is easily found in the catalogues of commercial enzyme suppliers. Thirty-three pages are devoted to a "theoretical study of the fraction of a long-chain DNA that can be incorporated in a recombinant DNA partial-digest library" and the representation of DNA sequences in such libraries. I don't know anyone who uses this information on a practical basis; moreover, the few qualitative aspects worth knowing are buried among pages of mathematical formulae.

Members of my laboratory found Per-

bal's book much harder to use than its competitor. One problem is that the book is bound in a conventional fashion, as compared to the earlier manual which comes as a spiral notebook. This seemingly trivial difference is actually of practical importance because Maniatis *et al.* lies flat and is thus easy to read on a laboratory bench, its natural habitat. Another problem is that the experimental protocols appear in large print, whereas explanatory paragraphs appear in small print. This is visually uncomfortable, and the large print for the protocols coupled with the small page size means that many techniques are spread out over a number of pages.

Thus it seems to me that the Maniatis *et al.* manual is still the best around, and the revised version should be even better. □

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Genesis of man

Harry V. Merrick

African Archaeology. By David W. Phillipson. Cambridge University Press: 1985. Pp.234. Hbk £25, \$39.50; pbk £9.50, \$14.95.

OVER the past two decades the pace of prehistoric archaeological research in Africa has accelerated enormously. Although recent work frequently has not produced as visually spectacular discoveries as in other parts of the world, it has been of the first order of importance in elucidating earlier human behaviour, especially during the Stone Age. Far from being a culturally stagnant cul-de-sac during the course of human evolution, Africa has often displayed a technological precociousness that would have astounded prehistorians 20 years ago.

In this book Dr Phillipson has brought together the new findings with earlier research to produce a very readable, relatively non-technical survey of the current state of knowledge of African prehistory. It is both timely and urgently needed; the last comparable synthesis is now 15 years old and has been largely superseded by recent events.

African Archaeology is very much a traditional, narrative account of the main stages of human development in Africa, emphasizing subsistence patterns and general ways of life. Each stage is presented in a series of regional summaries, accompanied by illustrations of the principal sites and representative tool and pottery types. Fortunately, much of the nomenclature and the intricacies of stone tool classification, which have dominated chronological and cultural reconstructions in African prehistory, are

neatly bypassed using Grahame Clark's scheme of technological modes to describe levels of technological complexity without temporal connotations. Nonetheless, details of chronology and technology still loom large for the first two million years of prehistory recounted in the first third of the volume. However, for the past several millennia, and especially for the Sub-Saharan region, Phillipson combines the findings of archaeology, including results from his own considerable field work, with linguistic and historical evidence and oral tradition to produce a much fuller view of emerging African societies in each part of the continent. This is an approach which also encourages appreciation of some of the environmental, economic and social factors underlying the cultural diversity of modern African peoples.

The bold attempt to encompass over two million years of a continent's prehistory in a single volume has understandably necessitated a synoptic treatment of most of the more controversial issues in African prehistory. While the superficial treatment of these problems may be trivial in the broader context of a continent-wide survey, regrettably it represents a missed opportunity to convey the excitement in African archaeological research today and the increasing inclination towards problem-orientated research. That apart, the book is impressively comprehensive, well-referenced and concise. It will be of particular interest to a broad range of Africanists and archaeologists, and of value as an introductory textbook for courses which cover African prehistory and history. □

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New in paperback

- *Quarks, Gluons and Lattices* by M. Creutz. Publisher is Cambridge University Press, price is £7.95, \$12.95. For review see *Nature* 309, 290 (1984).
- *From Folk Psychology to Cognitive Science: The Case Against Belief* by Stephen Stich. Publisher is MIT Press, price is \$8.95, £8.95. For review see *Nature* 309, 815 (1984).
- *Ontology and Phylogeny* by Stephen Jay Gould. Publisher is the Belknap Press of Harvard University Press and the price is \$8.95, £8.25.

Of the highest order

W.C. McGrew & J.R. Anderson

The Natural History of the Primates. By J.R. and P.H. Napier. *British Museum (Natural History)*: 1985. Pp.200. £15. To be published in the United States later this year by MIT press.

Primates in Nature. By Alison F. Richard. W.H. Freeman: 1985. Pp.558. Hbk \$27.95, £32.95; pbk \$17.95, £13.50.

In 1967, the Napiers' *A Handbook of Living Primates* appeared and immediately became the standard reference source for primatologists. Now the same authors have written another, slimmer reference work. There are several similarities between the two books: in each the bulk of the text is made up of potted profiles of primates arranged by genus, although the proportion given over to these has dropped in the new book, and some of the photographs and figures are the same as before. The differences, however, suggest that *The Natural History of the Primates* is aimed at a wider, more popular audience. New features are the inclusion of colour plates, a more spacious, double-columned lay-out and use of British as well as metric measures, while there is minimal citation of sources and only a short reference list. The writing is lively and clear, and the last chapter on human evolution packs a lot into only ten pages.

But the book does not fare so well in its stated role as a textbook. The content is sometimes repetitive (for example, three times, on pp. 19, 31 and 53, we are told that marmosets have claws and not nails) and is also somewhat old-fashioned in places. Key advances in the sociobiology and behavioural ecology of primates made over the past 15 years are rarely mentioned. Thus, for example, predators on primates are described as "beneficial" in helping to maintain the prey species as a "balanced viable population". Overall, this is a book which will better serve the keen amateur than the student or professional.

By contrast *Primates in Nature* is remarkable for its breadth of approach and the depth of ecological information provided; it deserves to become a cornerstone in the primatologist's library. One of the author's aims was to treat primates "as mammals rather than as our closest living relatives", that is to illuminate ecological generalities as well as features particular to primates, as in the adaptations of certain langurs and macaques to the problems of living in seasonally harsh temperate zones. Such comparisons do not dominate the book, however. Apart from a discussion of social organization in African hunting dogs and lions, non-primates take a back seat in Chapters 4-10 which concentrate on what is and is not

known about primates: as individuals (feeding, diet, reproduction), populations (demography), groups (social organization) and communities (interspecific relationships). The final chapter compares the position of primates and non-primates in ecological communities.

Dietary constituents and their distribution in plants are described lucidly in Chapter 4, followed by accounts of the feeding regimes of various species and of some of the corresponding morphological adaptations, notably dental and gastrointestinal. Some may feel that vertebrate prey are dismissed rather casually, for example in view of known seasonal and individual variations in meat-eating in chimpanzees. The same could be said for insectivory: while specialist insectivores

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Demidov's Bush-baby taken from Monkeys and Apes by Gotthard Berger and recently published by Arco. The price is \$24.95.

get their fair share of attention, insect-eating by non-specialists is passed over more quickly.

The author has adopted a case-history approach in describing aspects of primate ecology, illustrating a number of topics such as reproductive parameters, demographic patterns, social organization and so on with examples drawn from a range of species. This provides a broad perspective on primates as an order, and it is good to see prosimians and callitrichids given equal place alongside their larger and better-known counterparts. Anyone studying a field-site with more than one species of primate will benefit from reading Chapter 10 on sympatry, competition and the niche.

Primates in Nature does not include many data on social behaviour, for instance on grooming rates and on mother-offspring interaction. But it does contain solid background reading, a glossary of ecological terms and a wealth of ideas for preparing a primate field study, all in highly readable style. □

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Got that loving feeling

Michael Shepherd

Aphrodisiacs: The Science and the Myth. By Peter V. Taberner. Croom Helm/University of Pennsylvania Press: 1985. Pp.275. £19.95, \$22.50.

WHAT is an aphrodisiac? According to Peter Taberner, it is, strictly speaking, an exciter of lust; more generally, "anything which, by any means, increases the capacity for sexual enjoyment". With this definition he has given himself a broad canvas on which to sketch a readable, fact-filled review that roams over the wilder shores of sexuality.

As a professional pharmacologist the author is well qualified to discuss the properties and adverse effects of both the relatively small number of sexual stimulants in the medical pharmacopoeia and of the drugs of abuse which are used for their supposedly aphrodisiac properties. These constitute, however, no more than a small proportion of some 500 animal, vegetable and mineral substances listed in one of the appendices which have been or are being used as putative aphrodisiacs and to which reference is made in the text.

As its title indicates, the book is concerned with myth as well as science, with witchcraft and wormwood as well as bromocriptine and brain stimulation, with anthropology and medical history as well as the endocrinology and neurophysiology of sexual behaviour. The attainment of heightened sexual satisfaction ranks with gold, immortality and happiness as one of mankind's long-standing chimeras; as Havelock Ellis pointed out 80 years ago, "the early history of this subject is more or less inextricably commingled with folklore practices of magical origin". It still is, and Taberner has legitimately dipped into the rich fields of ancient tradition, magic, herbalism and quackery to provide many telling illustrations of human hopes and credulity, though pornography is unaccountably absent from the index.

A final chapter is devoted to aphrodisiacs of the future, and deals with such possible forerunners of a brave new world as pheromones, aromatherapy and cerebral stimulation. All a long way from the Act Two duet of Donizetti's *L'Elisir d'Amore*, in which the heroine Arina and the mountebank Dulcamara sing of the rival claims of love philtres and female charms, agreeing in the end that no potion is a substitute for genuine feeling. But perhaps they are singing of something other than Aphrodite. □

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Lots of GUTs

D.H. Perkins

Fifth Workshop on Grand Unification.

Edited by Kyungsik Kang, Herbert Fried and Paul Frampton. *World Scientific*/Wiley: 1984. Pp. 538. \$59.50, £51.75.

THE gravitational force between two protons is 10^{-38} of the electrical force. Where does such an odd number come from? Understanding the interrelation between the fundamental interactions of nature and their possible unification has always been a primary goal of the physicist. Regular conferences and workshops are devoted to the subject, the one in 1984 at Brown University being the fifth in a present series. This book contains the record of the meeting.

The first, and arguably the greatest intellectual leap was taken by Newton 300 years ago, when he proposed that the same universal gravitational force that made the apple fall also held the planets in orbit about the Sun. The next was the unification of electricity and magnetism by Maxwell in 1869. The third was the proposed unification of the weak and electromagnetic interactions by Salam, Weinberg and Glashow in the late 1960s which predicted the existence of the massive bosons W and Z, discovered at CERN two years ago.

Grand unification theories (GUTs) are a proposed further stage, originally identified with unification of the strong quark-quark force with the electroweak, but more recently taken to include gravity as well — thus encompassing all the known interactions in nature.

Maxwell's theory of electromagnetism was quickly verified by the detection of electromagnetic waves by Hertz, and the electroweak theory by the discovery of neutral weak currents in 1973 and the W and Z ten years later. However, despite early promise — the correct prediction of the value of the electroweak mixing angle — there is as yet hardly any experimental support for GUTs. The theory occurs in several versions, all of them making several predictions, none of them so far borne out by experience.

One of the early predictions was that of proton decay. This was not a new idea. Sakharov, in the context of the big bang model of the Universe, had pointed out in 1966 that the mechanism required to generate the huge preponderance of matter over antimatter in the Universe must also be responsible for proton decay at some level. Since 1973, GUTs had predicted a lifetime of around 10^{30} – 10^{33} yr, that is within experimental reach of huge (1 kiloton) detectors.

Papers in the present volume describe results of four experiments carried out deep underground in three continents. Their main achievement has been to ex-

clude one version of GUT: the lower limit on the lifetime for decay of a proton to a positron plus a neutral pion exceeds 10^{32} yr, more than an order of magnitude longer than the prediction. Needless to say, the other versions of GUTs make less definite predictions and so are harder to exclude. Yet all the experiments found candidates for other decay modes at 5–10 per cent of the total event rate, which is dominated by the atmospheric neutrino background. Finding out whether (or how) protons decay is going to be a long experimental struggle, and the question is hardly likely to be resolved by the tenth or even the fifteenth workshop in the series.

Another prediction of some versions of GUT is that leptons are not conserved and

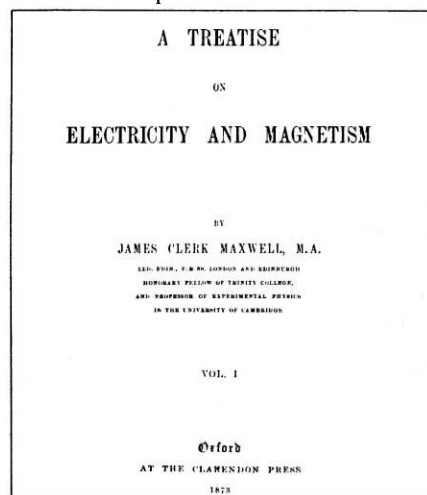
plus re-heat" version of the big bang is that the density of monopoles can be reduced to an acceptable number (one per galaxy, for example). Again, and just as in the case of neutrino oscillations, early possible signals have not been repeated and the most recent and sensitive experiments, seeking to detect monopoles through their ionization or currents induced in superconducting loops, find no evidence for their existence. Astrophysical limits on monopole flux, although inherently less reliable, set even more stringent flux limits.

Grand unified theories incorporating supersymmetry, in which it is postulated that all bosons are mirrored by fermion partners and vice versa, have become fashionable in recent years. None of this host of supersymmetric partners has so far been detected. In *pp* collider data at CERN over the past year, a few mysterious events (monojets) with large missing energy might be consistent with production of gluinos (the fermion counterparts of the gluons mediating the interquark force) — or equally with something else. Latest rumours are that even this possible signal may be vanishing.

Finally, theorists have proposed supergravity models, some of them based on the ideas of Kaluza and Klein of more than half a century ago. These start off with Einstein's theory in many dimensions, all but four of which are compacted and unobservable but which can be interpreted as the other fields (electromagnetic, strong and so on).

This volume gives a blow-by-blow account of the present status of the experimental and theoretical work in this most fundamental area of science. The hard core of practical evidence gives strong support for the old-hat "standard model" — of quark and lepton constituents, of the gauge models of the strong and electroweak interactions — and that is a solid achievement. The standard model is however somewhat ugly and theoretically unattractive because we cannot understand the huge range of particle masses, from less than 10 eV for neutrinos, through 10^{11} eV for the W and Z bosons, to 10^{28} eV for the Planck mass. So the theorists have gone off into what appears to many experimentalists to be esoteric wildernesses which have a beauty and symmetry of their own, and one of which might even provide the final answer. The main problem is that experiments in particle physics are very expensive and complex, and take five to ten years from first inception to final results. Those running now were inspired by the now out-dated theory of a decade ago. In short, we have lots of ideas and questions but, so far, hardly a real clue. □

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1873 — Maxwell publishes his work on the unification of electricity and magnetism.

that the different flavours of neutrino may transform one into the other. Historically, this was one possible way of accounting for the fact that the flux of electron-neutrinos from the Sun is a factor of three smaller than expected. Numerous attempts over the years to detect this flavour mixing — resulting in oscillations, for example in the electron-neutrino flux as a function of distance from a nuclear reactor — have all failed. With the exception of a single Russian experiment on tritium β -decay, the interpretation of which is open to question, all results to date are consistent with zero mass neutrinos and zero mixing. Four articles in the volume are devoted to recent results on double β -decay (setting upper limits on the neutrino mass) and on oscillation experiments at reactors and accelerators.

A third major prediction of GUTs is the existence of magnetic monopoles. These were originally invented by Dirac over 50 years ago, as a way of "explaining" quantization of electric charge. In GUTs however, the charge quantization follows from the construction of the theory, and the result is that massive elementary monopoles, of mass 10^{16} GeV or 10^{-8} g, absolutely have to exist. In the original big bang model of the Universe, enormous numbers of monopoles would be predicted. A free bonus of the "inflationary

Intensity-dependent quasi-periodic oscillations in the X-ray flux of GX5-1

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The X-ray flux of the bright galactic bulge source GX5-1 shows intensity-dependent quasi-periodic oscillations between ~20 and ~40 Hz, appearing as a broad peak in the power spectrum whose centroid frequency, width and integrated excess power strongly depend on the source intensity. The strength and steepness of low-frequency noise present in the power spectra below 15 Hz also depend on the source intensity. No evidence is found for coherent X-ray pulsations between 0.5 and 2,000 Hz. We discuss possible mechanisms to explain these new phenomena.

NEUTRON stars in low-mass X-ray binaries have long been suspected to have been spun up to periods in the millisecond range. Several searches for such short rotation periods in low-mass X-ray binaries have been made but none has been successful (refs 1-4 and J. Fleischman, S. Rappaport, D. Strahs and W. Lewin, personal communication). We have renewed the search using the medium energy detectors of the X-ray observatory Exosat. One of the first sources we observed was the bright bulge source GX5-1, in which we found no periodic pulsations but, unexpectedly, we discovered quasi-periodic oscillations. These oscillations are the subject of this report; some preliminary results have already been reported⁵.

Observations

We observed GX5-1 (4U 1758-25) with the medium-energy detector⁶ (ME) and the gas scintillation proportional counter⁷ on Exosat. The observations started on 18 September 1984 11:50 UT and lasted for ~8 h. We shall discuss 1-18-keV data obtained with the Ar/CO₂-filled proportional counters of the medium-energy detector; the counters were co-aligned with a total effective area of 1,500 cm². The data were obtained in 2-s blocks, each containing 8,192 samples (time resolution ~0.25 ms). Within each of the 1,623 blocks, the data are continuous with a negligible 'dead time' of ~1% and between two consecutive data blocks there is a gap of 15 s. The data contain no spectral information.

Figure 1 shows the counting rate as a function of time. The data include an approximately constant background of ~80 c.p.s. The sharp drop near $t = 17,000$ s is caused by a change ('trim') in the satellite pointing direction. The reason for this trim was the high counting rate, at which detector damage was feared. Before the trim, the collimator efficiency was 93%, whereas afterwards it was 65%.

For the average spectral shape, as measured with the gas scintillation proportional counter, 1 c.p.s. from the ME (pre-trim collimator efficiency) corresponds to $\sim 9.2 \times 10^{-12}$ erg cm⁻² s⁻¹ (1-18 keV), or ~ 0.3 μ Jy (2-11 keV). The source flux (~ 700 - $\sim 1,100$ μ Jy) and its variability on a timescale of hours are similar to those encountered previously⁸⁻¹². All count rates described here have been reduced to the pre-trim collimator efficiency.

Analysis and results

To investigate the variability of GX5-1 on timescales between 0.5 ms and 2 s, we estimated the power spectrum by calculating (via a fast Fourier transform (FFT) algorithm) the Fourier amplitudes of the average-subtracted signal in each 2-s block

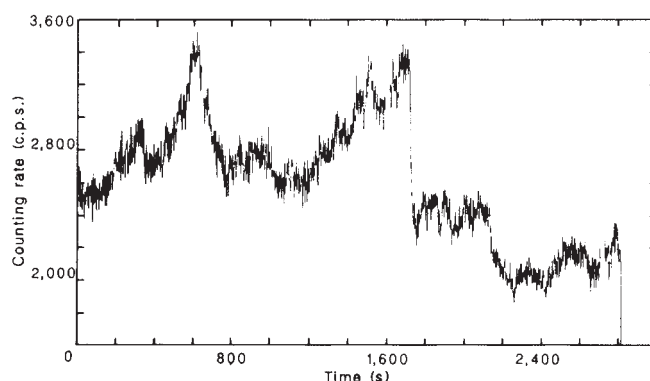


Fig. 1 Average counting rates in 2-s time intervals. Zero time corresponds to 18 September 1984, 11:53:35 UT. The sharp drop near $\sim 17,000$ s is the result of a change in the pointing direction of the satellite (see text).

separately. The power spectra of single data blocks are too noisy to allow meaningful study. However, the average of these power spectra clearly shows three components: (1) low-frequency noise below 15 Hz (the power increases towards lower frequencies); (2) a strong broad peak with a centroid frequency near 30 Hz; and (3) a flat spectrum above 100 Hz, consistent with Poisson noise.

No evidence was found for coherent pulsations in the X-ray flux. The 99% confidence upper limit to the pulsed fraction of coherent pulsations in the 50-400-Hz range is 0.3%; in the 400-2,000-Hz range it varies between 0.6 and 2.5%, depending on frequency. In calculating these values, we followed the procedures described by Leahy *et al.*³ and took into account possible Doppler shifts resulting from orbital motion of the source and the satellite.

Power density estimates, using conventional FFT techniques, may be in error because of low-frequency 'leakage', if a steep low-frequency ('red') noise component is present¹³. The power-law slope of the red noise in our data is considerably below the critical value of 2, above which problems may occur. Low-frequency leakage is therefore not expected to affect our analysis. This was confirmed by tests on artificial red-noise data and by repeating the analysis of the 2-s blocks after 'detrending' each of them by subtracting a low-order polynomial.

The properties of the broad peak in the power spectrum (centroid frequency, width and integrated excess power) and the low-frequency noise (slope, and integrated excess power) strongly depend on source intensity. In Fig. 2a, b we show the

observed power spectra as a function of time and source intensity, respectively. The broad peaks in the power spectra are seen in Fig. 2a as a dark 'ribbon'; the low-frequency noise is visible at the bottom. The centroid frequency ν_c is clearly correlated with the source intensity (Fig. 2a). Also, both the quasi-periodic oscillations and the low-frequency noise diminish at high source intensity. The dependence of ν_c on source intensity is approximately linear (Fig. 2b). This dependence is not affected by the $\sim 30\%$ drop in counting rate caused by the 'trim' near $t = 17,000$ s (Fig. 1). A power-law dependence, however, is also consistent with the data.

The fact that ν_c depends on source intensity and not on the counting rate in the instrument convinced us that the peak in the power spectrum is related to a phenomenon in GX5-1 and is not an instrumental effect or an artefact of the analysis. In this context we mention that background data obtained immediately before and after our observation with the same time resolution, and observations of several other sources of similar brightness, did not show a peak in the power spectrum.

As far as we can tell at this stage of the analysis, all variable properties of the power spectra depend on the source intensity only. To describe the power spectra quantitatively, we performed least-squares-fits to the averages of power spectra selected from six source-intensity intervals. As a fitting function we used

$$P(\nu) = A + B e^{-\gamma\nu} + C[(\nu - \nu_c)^2 + (\lambda/2)^2]^{-1} \quad (1)$$

where P is the power density and B and C are normalization constants. The first term on the right-hand side (a constant) describes the white-noise component. In our adopted normalization³ one expects $A = 2$ for poissonian noise. The low-frequency noise is well described by an exponential function (second term in equation (1)). A power law does not adequately fit the data.

We used a Lorentz profile with a full-width at half maximum, λ , and centroid frequency ν_c (third term in equation (1)) to describe the peak. We also tried to fit the peak with a gaussian profile; this did not give better power-law fits to the low-frequency data. (The width and integrated excess power of the peak are then 20–30% lower.)

The fits of expression (1) to the averaged power spectra (first 512 frequencies) have a reduced χ^2 between 1.03 and 1.17 for 506 degrees of freedom. The variance of the power estimates was taken as $4/N$, N being the number of power spectra averaged³. Examples of the fits are shown in Figs 3 and 4. For decreasing source intensity, ν_c decreases, the peak becomes narrower and the low-frequency noise component steepens.

Figure 5 shows plots of the numerical results (listed in Table 1). The centroid frequency of the peak (Fig. 5a) shows a strong, approximately linear correlation with source intensity. The peak width (Fig. 5b) and the steepness of the low-frequency noise spectrum (Fig. 5c) show an approximately monotonic dependence on the source intensity. The integrated excess power of both the peak (Fig. 5d) and the low-frequency noise (Fig. 5e) show a maximum, roughly halfway in the observed intensity range.

The width and integrated excess power of the peak are 30–50% lower than shown in Fig. 5 (b, d) if we choose a power-law description of the low-frequency noise (both for a lorentzian and a gaussian peak profile). The centroid frequency is very insensitive to changes in the fitting function.

In principle, the broad peaks in the averaged power spectra could result from the averaging out of very narrow peaks which occur at different frequencies in the power spectra of the individual 2-s data blocks. However, the peak remains broad when the data are analysed in intervals as short as ~ 100 s.

We shall assume that the averaged power spectra are representative of those of the individual 2-s blocks. The results can then be interpreted as resulting from a signal that shows quasi-periodic oscillations of which the typical period decreases from 50 to 25 ms and the coherence time (defined as the e folding time of autocorrelation function) from 75 to 25 ms when the source intensity increases by $\sim 30\%$. The signal also contains low-frequency noise to which the contribution of slower vari-

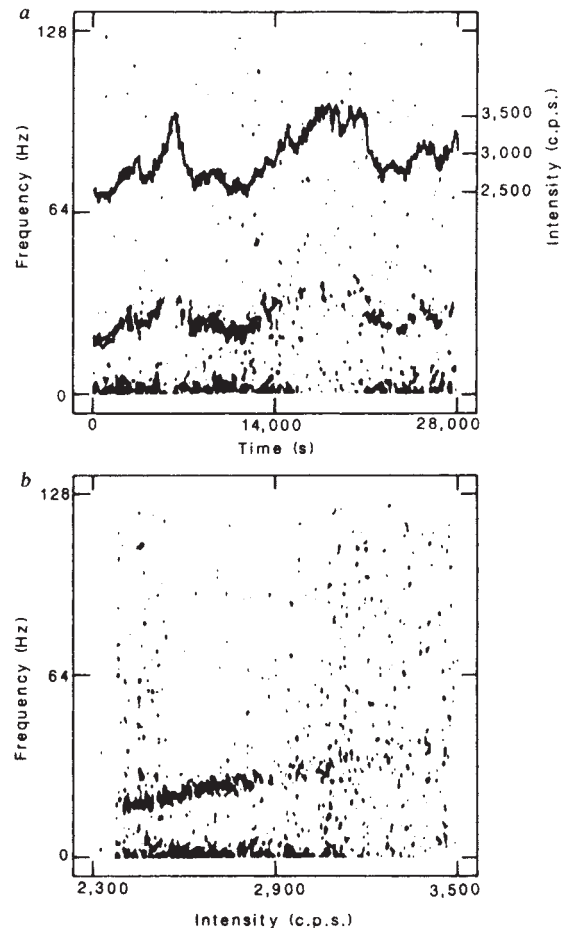


Fig. 2 a, Display of the temporal variation of the power spectra. The frequency is in the vertical direction (left-hand scale). A vertical cross-section of the display is a grey-scale coded representation of a power spectrum. The darker the shade the higher the power it represents. To conserve the quality of the picture, the original display of 512×512 pixels has been smoothed with a gaussian profile with $\sigma = 1.3$ pixels. The variable broad peak in the power spectra is visible as a dark 'ribbon'; the low-frequency noise is visible at the bottom. Also shown is the intensity of GX5-1 (right-hand scale). The peak frequency depends strongly on source intensity. Also, notice that both the quasi-periodic oscillations and the low-frequency noise diminish at high source intensity. b, Display (see a) of the power spectra as a function of source intensity (reduced to pre-trim collimator efficiency). The peak frequency is approximately linearly related to source intensity.

ations becomes weaker for increasing source intensity and of which the strength (integrated excess power) depends on the source intensity in a way very similar to that of the peak.

The noise process that underlies the quasi-periodic character of these oscillations is not strongly constrained by our present analysis; the counting rates are too low to allow us to follow individual wave trains. It could be the result of (see, for example, ref. 14): (1) a superposition of randomly excited damped harmonic oscillators; (2) random phase variations of a periodic wave train; (3) the superposition of closely spaced periodic oscillations whose frequencies are distributed over a finite interval. It is also conceivable that the broad peak is the result of a dependence of the peak frequency on the X-ray photon energy, which is smeared out because of the absence of energy resolution in our data.

Discussion

Intensity-dependent quasi-periodic oscillations, such as those reported here, have not been observed previously (millisecond X-ray oscillations lasting of the order of 10 s have been reported^{2,15} which are probably unrelated). The variations in frequency of the oscillations clearly exclude the possibility that

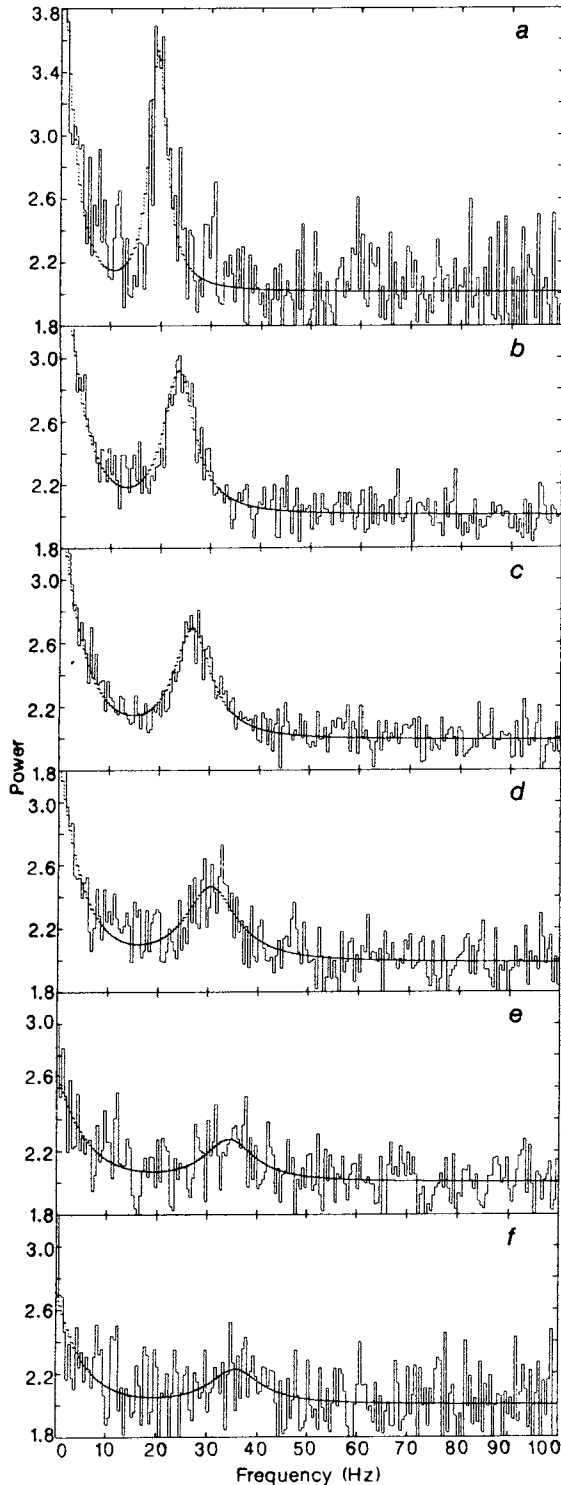


Fig. 3 Linear displays of average power spectra of GX5-1 in six different source-intensity intervals. *a*, 2,277–2,486; *b*, 2,486–2,695; *c*, 2,695–2,904; *d*, 2,904–3,113; *e*, 3,113–3,322; *f*, 3,322–3,531 (c.p.s.). The average has been taken over the power spectra of individual 2-s intervals (see text). Fits of equation (1) to the data are also shown. Notice the change of centroid frequency and width of the peak as a function of source intensity.

they represent the rotation period of the compact object.

Quasi-periodic oscillations on timescales of the order of tens of seconds are a common phenomenon in cataclysmic variables^{14,16,17}. Of particular interest are the optical oscillations seen during some dwarf-nova outbursts which show a positive correlation between frequency and (optical) flux^{14,18,19}. Apart from the $\sim 10^3$ times longer period and the different energy band, these oscillations differ from those in GX5-1 by: (1) the generally one to three orders of magnitude larger coherence; (2) the

positive correlation between coherence time and source intensity¹⁹ (negative in GX5-1, see Figs 3, 4, 5*b*); and (3) the much weaker dependence of frequency on optical flux.

Models proposed for these dwarf-nova oscillations include inhomogeneities rotating with the inner accretion disk^{20,21}, or with the white dwarf envelope²²; disk oscillations^{23,24}; oscillations in the atmosphere of the white dwarf^{25,26}; and various instabilities in the accretion flow (for example 27–29; for reviews see refs 14, 18). We examine some of these models in the context of our new findings for GX5-1, assuming that the compact object is a neutron star; in the case of cataclysmic variables the compact object is a white dwarf.

The dependence of the frequency of the oscillations on the source intensity in GX5-1 is strong ($d \log \nu_c / d \log I \sim 2$). The accretion-flow instability models of Langer²⁸ and Livio²⁹ can be excluded on this ground alone. The model of King²², which involves magnetic loops anchored in a differentially rotating envelope of the compact object, predicts the correct sense for the correlation of intensity with frequency and coherence of the oscillations, but does not make quantitative predictions.

Non-radial *g*- or *r*-mode oscillations of the neutron star envelope could have periods in the range observed by us^{30,31}. Proposed excitation mechanisms for these oscillations are thermonuclear flashes³² (see also ref. 33) and sudden accretion events³⁴. However, X-ray bursts have never been observed from GX5-1. It is unclear whether, with a mass-accretion rate varying by $\sim 30\%$, the second mechanism can be considered viable.

In Bath's²⁰ model for dwarf-nova oscillations, the oscillations originate from inhomogeneously distributed matter ('blobs') orbiting the compact object with the Kepler frequency at some preferred radius, in particular that of the magnetosphere, r_m . In the case of spherical accretion, r_m is given by^{35,36}

$$r_m = (2.9 \times 10^8 \text{ cm}) \mu_{30}^{4/7} m^{1/7} R_6^{-2/7} L_{37}^{-2/7} \quad (2)$$

Here μ_{30} is the magnetic moment of the compact object in units of 10^{30} G cm^3 , m its mass in units of solar masses, R_6 its radius in units of 10^6 cm and L_{37} the X-ray luminosity in units of $10^{37} \text{ erg s}^{-1}$. In our further discussion we assume $m = R_6 = 1$.

The keplerian frequency ν_K at the magnetospheric radius depends on the X-ray luminosity according to

$$\nu_K = (0.37 \text{ Hz}) L_{37}^{3/7} \mu_{30}^{-6/7} \quad (3)$$

With $\nu_K \sim 30 \text{ Hz}$ and $L_{37} \sim 10$, one infers $\mu_{30} \sim 2 \times 10^{-2}$ or, equivalently, a surface magnetic field strength $B_0 \sim 2 \times 10^{10} \text{ G}$ and $r_m \sim 160 \text{ km}$. The relation between the frequency of the quasi-periodic oscillations and the luminosity for GX5-1 is qualitatively as predicted by equation (3). However, as mentioned above, the observed value for the exponent is about five times the predicted value of $3/7$, and this may indicate that this model²⁰ is not applicable here. Alternatively, disk oscillations^{23,24} could occur near, for example, the magnetospheric radius. The periods of disk oscillations are of the order of the Kepler periods³⁷; the oscillations would then occur at a radius of $\sim 10^2 \text{ km}$. The quasi-periodic oscillations in the X-ray flux could, for example, be produced by intermittent occultations of the X-ray source by the orbiting blobs or by the oscillating disk.

Warner²¹ suggested that the observed frequency, ν_c , of the dwarf nova oscillations is the beat frequency of the (keplerian) disk frequency, ν_K , at the magnetospheric radius and the rotation frequency, ν_s , of the white dwarf. Alpar and Shaham^{38,39} suggest the same idea to explain the oscillations in GX5-1. For the case of the neutron star this leads to

$$\nu_c = (0.37 \text{ Hz}) \mu_{30}^{-6/7} L_{37}^{3/7} - \nu_s \quad (4)$$

The dependence of ν_c on luminosity can then be made stronger at will by a proper choice of the value of ν_s . A fit of relation (4) to our data (dashed curve in Fig. 5*a*) yields $(\nu_s)^{-1} = 10.5 \pm 0.4 \text{ ms}$ and $B_0 = (6.3 \pm 0.3) \times 10^9 (D/10 \text{ kpc}) \text{ G}$; here D is the source distance. The magnetospheric radius is $\sim 60 \text{ km}$.

The derived magnetic field strength depends strongly on whether the blobs are seen at the magnetospheric radius, r_m (equation (2)), or at some other radius. According to Ghosh and Lamb⁴⁰, for disk accretion, the appropriate magnetospheric

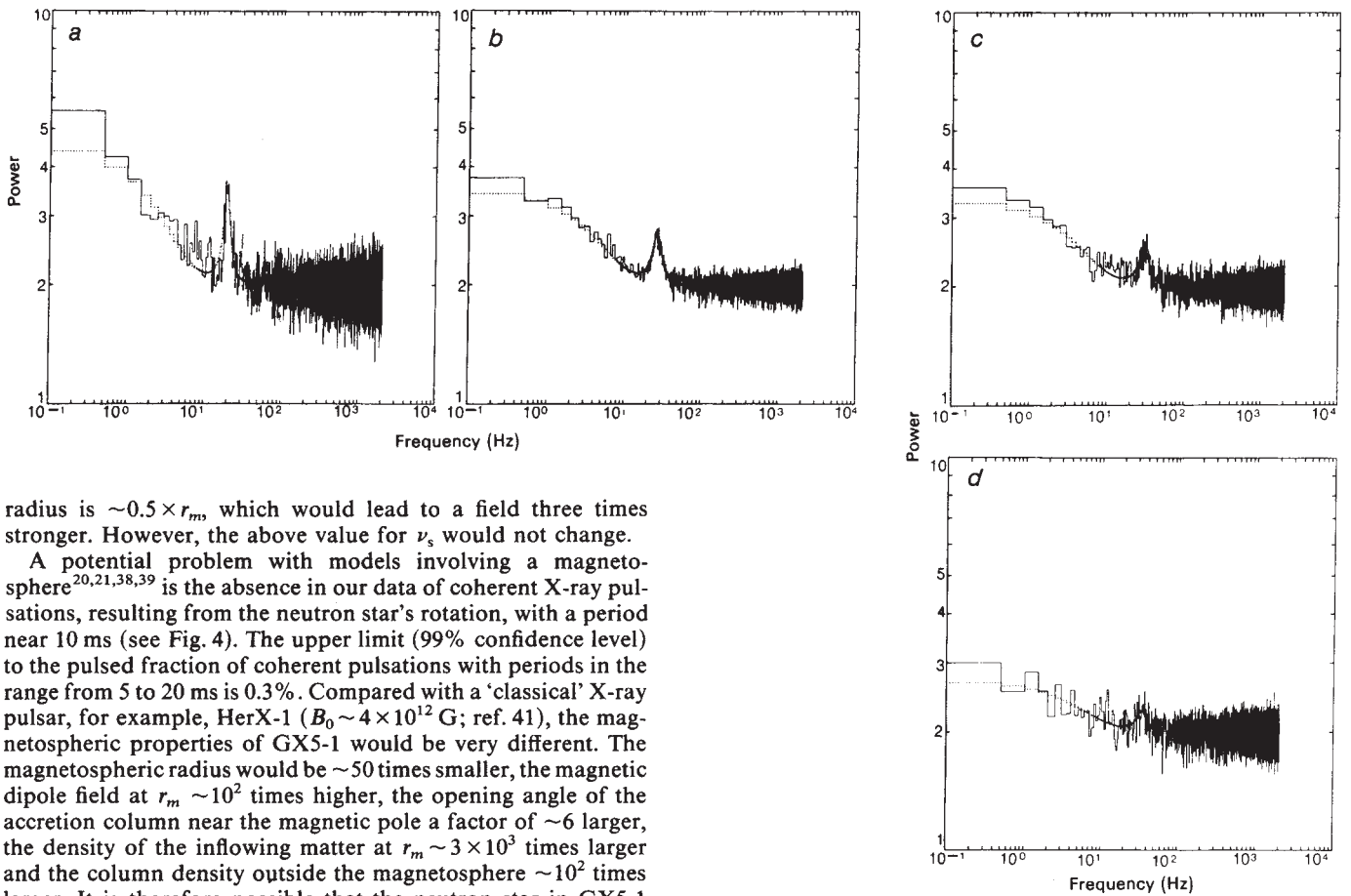


Fig. 4 Logarithmic displays of average power spectra in four different source intensity intervals (see Fig. 3 legend). a, 2,277–2,486; b, 2,695–2,904; c, 2,904–3,113; d, 3,113–3,322 (c.p.s.). The dependence of the low-frequency noise on source intensity is seen more clearly than in Fig. 3.

radius is $\sim 0.5 \times r_m$, which would lead to a field three times stronger. However, the above value for ν_s would not change.

A potential problem with models involving a magnetosphere^{20,21,38,39} is the absence in our data of coherent X-ray pulsations, resulting from the neutron star's rotation, with a period near 10 ms (see Fig. 4). The upper limit (99% confidence level) to the pulsed fraction of coherent pulsations with periods in the range from 5 to 20 ms is 0.3%. Compared with a 'classical' X-ray pulsar, for example, HerX-1 ($B_0 \sim 4 \times 10^{12}$ G; ref. 41), the magnetospheric properties of GX5-1 would be very different. The magnetospheric radius would be ~ 50 times smaller, the magnetic dipole field at $r_m \sim 10^2$ times higher, the opening angle of the accretion column near the magnetic pole a factor of ~ 6 larger, the density of the inflowing matter at $r_m \sim 3 \times 10^3$ times larger and the column density outside the magnetosphere $\sim 10^2$ times larger. It is therefore possible that the neutron star in GX5-1 cannot be seen directly because it is obscured by plasma. It is also conceivable that a special geometry of 'X-ray beams' may hide the signature of the neutron star spin, or that the magnetic and rotation axes are co-aligned.

Despite the fact that the beat-frequency model^{21,38,39} has been introduced in a rather *ad-hoc* fashion to account for the observed relation between frequency and intensity, the inferred rotation period and magnetic-field strength of the neutron star fit well³⁸ into an existing evolutionary framework for the galactic bulge sources^{42,43}, which links these objects to the 6-ms radio pulsar⁴⁴ PSR1953+29.

In this evolutionary model^{42,43}, neutron stars in bright galactic bulge sources are spun up to millisecond rotation periods because of accretion from an evolved companion, and the X-ray

sources evolve into wide binary radio pulsars^{45–47}. (That a spun-up neutron star with a relatively weak magnetic field, in a binary, may later on show up as a fast radio pulsar was first suggested by Smarr and Blandford⁴⁸ and further elucidated in refs 49–53.) The rotation period of ~ 10 ms, as predicted by the beat-frequency model for GX5-1, is in accordance with the evolutionary model^{42,43}. The magnetic dipole field strength of PSR1953+29 ($\leq 2 \times 10^{10}$ G; refs 45, 54) is comparable to that predicted by

Table 1 Best-fit parameters of power spectra

N	97	425	464	288	208	128
I_{lim} (c.p.s.)	2,277–2,486	2,486–2,695	2,695–2,904	2,904–3,113	3,113–3,322	3,322–3,531
$\bar{I}$ (c.p.s.)	2,427	2,603	2,790	3,001	3,225	3,403
ν_c (Hz)	20.07 ± 0.15	24.38 ± 0.16	27.26 ± 0.2	31.25 ± 0.5	35.11 ± 1.0	36.35 ± 1.6
λ (Hz)	4.2 ± 0.6	7.6 ± 0.5	9.2 ± 0.7	12.6 ± 2.0	12.4 ± 4.0	12.6 ± 10.0
$P_{\text{peak}} (10^4 \text{ (c.p.s.)}^2)$	2.47 ± 0.13	2.89 ± 0.13	3.27 ± 0.2	3.33 ± 0.4	2.03 ± 0.6	2.02 ± 1.0
$f_{\text{peak}} (\%)$	6.48 ± 0.17	6.53 ± 0.15	6.48 ± 0.2	6.08 ± 0.4	4.42 ± 0.7	4.18 ± 1.0
γ (s)	0.367 ± 0.04	0.247 ± 0.014	0.211 ± 0.014	0.216 ± 0.02	0.168 ± 0.04	0.188 ± 0.08
$P_{\text{LFN}} (10^4 \text{ (c/s)}^2)$	2.32 ± 0.15	2.65 ± 0.10	2.83 ± 0.12	2.72 ± 0.19	1.93 ± 0.25	2.15 ± 0.4
$f_{\text{LFN}} (\%)$	6.28 ± 0.2	6.25 ± 0.12	6.03 ± 0.13	5.50 ± 0.19	4.31 ± 0.3	4.31 ± 0.4
A	2.011 ± 0.009	2.004 ± 0.004	1.987 ± 0.004	1.983 ± 0.005	2.003 ± 0.007	2.002 ± 0.010
χ^2_ν (506 d.f.)	1.17	1.07	1.03	1.09	1.07	1.05

Results of least-squares fits of equation (1) to averaged power spectra taken from six source-intensity intervals. N is the number of individual power spectra (2-s data blocks) averaged, the values for I_{lim} indicate the limits in source intensity defining the intervals, and $\bar{I}$ is the average intensity within each interval. Peak centroid frequency ν_c , full-width at half-maximum λ , steepness parameter γ of the low-frequency noise and the white-noise level A are defined by equation (1). P_{peak} and P_{LFN} are the integrated excess power in the peak and in the low-frequency noise, respectively. These numbers are the integrals ($\nu = 0$ to ∞) of the third and the second term, respectively, of the fits of equation (1), to the power spectra, normalized to assign a power a^2 to a sinusoidal modulation with amplitude a . f_{peak} and f_{LFN} are the corresponding relative amplitudes. Quoted errors correspond to the extreme limits of the $\chi^2_{\text{min}} + 1$ error contours.

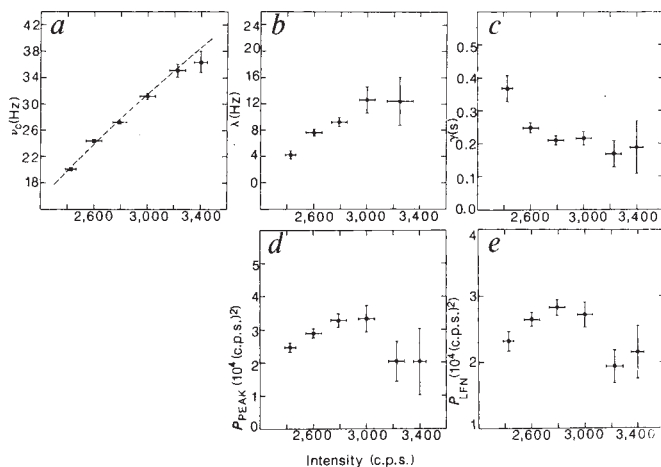


Fig. 5 Parameters of the power spectra (see Table 1) as a function of source intensity. *a*, Centroid frequency ν_c of the peak in the power spectrum. The dashed curve is a fit of equation (4) to the points. Clearly, a linear relation would also be an excellent fit to our results. *b*, Full-width at half maximum, λ , of the peak in the power spectrum. In this panel the two highest intensity intervals have been combined. *c*, Steepness parameter, γ , of the low-frequency noise. *d*, Integrated excess power of the peak in the power spectrum. Note the striking similarity with *e*. *e*, Integrated excess power of the low-frequency noise. Note the striking similarity with *d*. Vertical error bars are 1σ single-parameter, and take correlations between parameters in the fit into account. Horizontal bars represent the standard deviations of the intensity distributions in each of the six chosen intervals.

the beat-frequency model for the neutron star in GX5-1. These coincidences make the beat-frequency model attractive, but they could well be accidental.

Magnetic fields of neutron stars probably decay on a timescale of $(2-5) \times 10^6$ yr⁵⁵ (but see ref. 56). Therefore, if the quasi-periodic oscillations in GX5-1 are related to a magnetosphere (with a magnetic field strength of the neutron star of the order of 10^{10} G), the age of the neutron star in GX5-1 is probably of the order of 5×10^7 yr. Van den Heuvel and Taam⁵⁷ pointed out that, in the above evolutionary model^{42,43}, neutron stars are most probably formed during the mass-transfer phase by the accretion-induced collapse of a white dwarf. The mass-transfer phase in systems with an evolved companion lasts only $\sim 10^8$ yr. Thus, neutron stars in such systems could be relatively young ($< 10^8$ yr). The mass-transfer phase in systems with low-mass unevolved companions can last up to $\sim 10^9$ yr. Thus in those systems the probability of finding a relatively young neutron star is small. Therefore, if magnetospheric models are correct and the above evolutionary scenario is applicable, one would expect that similar quasi-periodic oscillations in low-mass X-ray binaries may be observed preferentially in systems which have an evolved companion star.

Our data suggest that the low-frequency noise is a different manifestation of the same phenomenon that causes the oscillations (notice the similarity between Fig. 5*d* and *e*). If we speculate that the X-ray oscillations are the result of 'blobs' in the inner accretion disk, the low-frequency noise could perhaps represent the size distribution of the blobs, larger blobs contributing to the noise power at lower frequencies. The blobs would have to disappear when the source intensity is high (decline of low-frequency noise). In this scenario, we can derive a lifetime for the blobs of 0.1–0.2 s from the observed width of the peaks in the power spectra. This estimate is based on the assumption that during its lifetime a blob causes a wavetrain of constant amplitude and frequency. We observe (see Fig. 5*b*, *c*) that when the steepness of the low-frequency noise is at a maximum (many large blobs present), the peak in the power spectrum is relatively narrow (long blob lifetime). This is contrary to what would be expected if this lifetime is dictated by differential keplerian rotation⁵⁸.

Possibly there is a 'gating' mechanism which allows incoming blobs (producing the low-frequency noise) to accrete in a piecemeal fashion (producing the quasi-periodic oscillations). This mechanism could then 'exhaust' the blobs, and now larger blobs may survive longer than smaller ones, consistent with our observations. A gating mechanism would probably fit in any of the above magnetospheric models^{20,21,38}.

Clearly, the scenarios and ideas discussed here are speculative. Any successful model must produce a physical mechanism explaining the observed properties of the quasi-periodic oscillations and the low-frequency noise, including their strong dependence on the source intensity.

We observed GX5-1 again for ~ 8 h on 29 April 1985. An analysis, performed the same day, showed that the quasi-periodic oscillations were again present. When this manuscript was in the final stage of typing, Dr W. Priedhorsky and his co-workers informed us that they observed (personal communication) similar phenomena from ScoX-1 as described here for GX5-1.

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Note added in proof: Models for the quasi-periodic oscillations along the lines suggested at the end of this article have been proposed by Alpar, Lamb and Shaham⁵⁹ and by Berman and Stollman⁶⁰. Intensity-dependent quasi-periodic oscillations have also been detected in CygX-2 (Hasinger *et al.*, IAU Circ. 4070), and quasi-oscillations have been demonstrated to be intensity dependent in ScoX-1 (M.v.d.K. *et al.*, IAU Circ. 4068). We point out that CygX-2 has an evolved companion, which supports our suggestion that the oscillations will be preferentially observed in such systems. We thank Fred Lamb for a useful discussion concerning the coherence time of the oscillations.

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How the geomagnetic field vector reverses polarity

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A highly detailed record of both the direction and intensity of the Earth's magnetic field as it reverses has been obtained from a Miocene volcanic sequence. The transitional field is low in intensity and is typically non-axisymmetric. Geomagnetic impulses corresponding to astonishingly high rates of change of the field sometimes occur, suggesting that liquid velocity within the Earth's core increases during geomagnetic reversals.

THE Steens Mountain (Oregon) reversed-to-normal polarity transition¹ is probably the most detailed record of a reversal of the geomagnetic field reported from a volcanic sequence. This Miocene reversal occurred 15.5 ± 0.3 Myr ago^{2,3} and can be correlated with the older boundary of marine magnetic anomaly 5 B2 (ref. 4). We have completed an extensive palaeomagnetic study of the Steens Mountain reversal to obtain a precise, detailed description of fluctuations in absolute palaeointensity of the geomagnetic field as it reverses. In the course of this study, we also obtained a more complete description of the directional changes during this reversal. These directional and palaeointensity data place new major constraints on the reversing Earth's dynamo.

Field and laboratory methods

We collected slightly over 1,000 oriented samples from three almost completely sampled sections, A, B and C, of the upper two-thirds of the Steens Basalt. Sections A and B are located only 1 km apart on Steens Mountain (42.63° N, 241.43° E) and are readily correlated by matching the transitional field directions. Taken together, these two sections span the end of the pre-transitional reversed period, the transition and the post-transitional normal period. Samples from section C (42.18° N, 240.07° E), located 130 km to the south-west of Steens Mountain, are reversed with the exception of those from the top flow, which display a transitional direction also observed on Steens Mountain¹ at the start of the reversal. The main eruptive centre was probably close to Steens Mountain, and the reversed part of Section C is believed to be older than the reversed zones sampled at the bottom of sections A and B, on the basis of the sequence of palaeomagnetic directions. The composite section obtained by combining sections A, B and C is 615 m thick and consists of ~120 distinct lava flows, which are numbered within each section from the top to the bottom.

The palaeodirection of the field was calculated from the remanence direction after either alternating field or thermal cleaning. After grouping successive flows with the same average direction of remanence, the Steens record consists of 55 directional groups (also numbered from the top to the bottom) of which 12 are reversed, 29 are transitional and 14 are normal (Fig. 1).

The absolute palaeointensity of the field was determined using the method of Thellier and Thellier⁵. Although time-consuming,

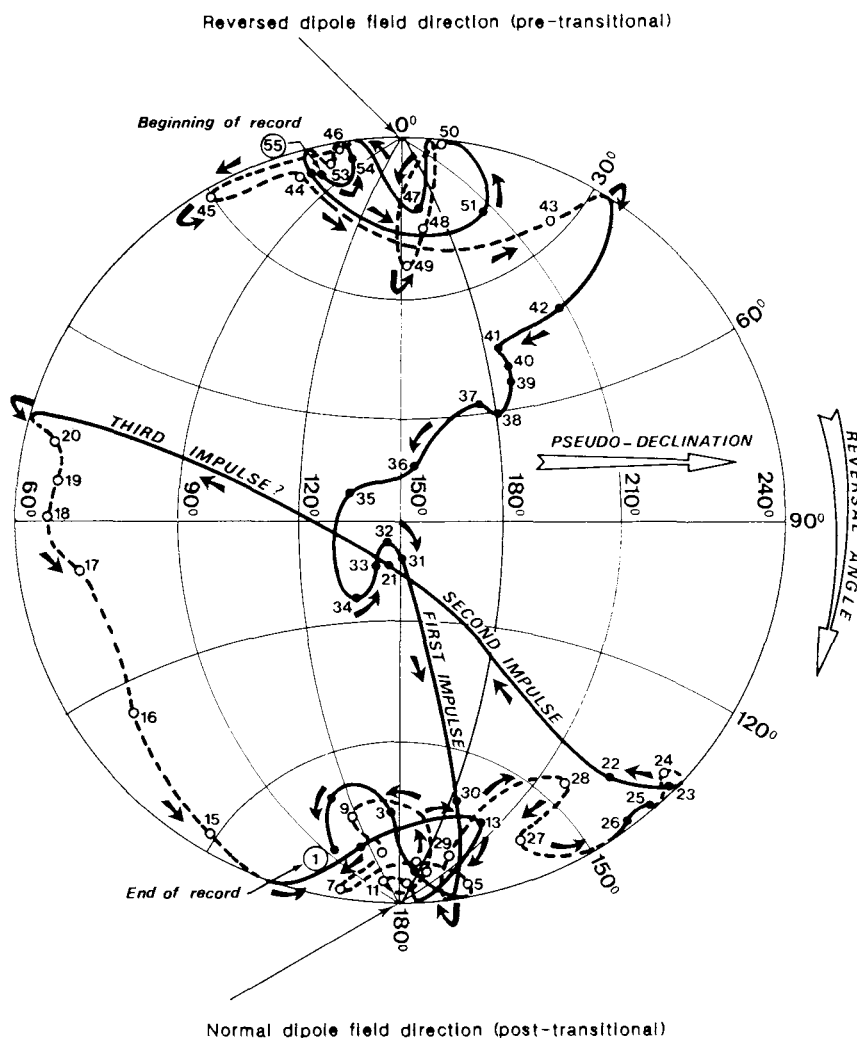
this method is preferable because it relies on experimentally well-established physical laws and a sound theoretical background. In addition, the method allows palaeointensities to be determined without the direct heating of samples to high temperatures required by other methods, which usually result in major magnetochemical changes. We selected 185 samples for palaeointensity investigations with low viscosity index, high Curie point and reversibility (or near-reversibility) of the strong-field magnetization curve versus temperature. These criteria proved to be pertinent. We obtained 157 useable palaeointensity values corresponding to 51 vectorial groups (group of successive flows with the same directions and palaeointensities).

Vectorial description

The transitional state of the geomagnetic field vector may be defined as corresponding to successive vectors of which either the directions or intensities (or both) are outside the range of secular variation observed in the adjacent pre- or post-transitional periods. Using this criterion, the end of the transition corresponds to directional group (DG) 15 whose direction and intensity are both transitional (Fig. 2). The beginning of the reversal was recorded by DG 43, which displays the first transitional palaeointensity (Fig. 2). However, the direction of the field at the same time was only 27° away from that of the reversed dipole (Fig. 1) and may be considered to be within the range of secular variation. Thus, transitional palaeointensities seem to have shortly preceded the transitional directions, which were recorded by the immediately following directional groups. In contrast, normal directions and intensities were recovered simultaneously at the end of the reversal.

Records of secular variation before and after the reversal were used to obtain a crude estimate of the duration of the transition. We assumed that these records are essentially complete because of the high extrusion rate needed to record the field reversal in such detail. A review of archaeomagnetic results⁶ shows that the angular rate of change of the virtual geomagnetic pole, when calculated from 2,000 yr BP to the present, is almost the same ($6 \pm 1^\circ$ per 100 yr) for areas as widely separated as Europe, North America and Japan. Assuming that this rate can be used for the Miocene, we found that the reversed and normal records in the Steens Basalt correspond to periods of 5,000 and 3,500 years, respectively. The corresponding accumulation rates of flows at Steens Mountain (sections A and B) are the same (43 ± 4 m per

Fig. 1 The Steens Mountain directional record. Stereographic projection (true angle) of the rotated field direction of the successive directional groups. Directions are rotated by 28.5° about the east-west horizontal axis to bring the dipole field directions coincident with the poles of the projection sphere³⁶. The reversal angle varies from 0° (reversed dipole direction) to 180° (normal dipole direction), and corresponds to lines of equal 'latitude' on the projection sphere. Lines of equal 'longitude' correspond to pseudo-declination, which is measured in the 'equatorial' plane from the projection of the north-seeking direction into this plane. A purely axisymmetrical field would have a pseudo-declination equal to 0 or 180° , which corresponds to near-sided or far-sided¹³ virtual geomagnetic pole paths, respectively. Directional group numbers are indicated near most of the circles representing the successive average field directions. With few exceptions (see Fig. 3), 95% confidence semi-angles are only a few degrees and could not be drawn on this diagram. Full circles and solid path are on the hemisphere of the projection sphere which contains the pole of projection (reversal angle 90° , pseudo-declination 150°); empty circles and broken path are on the opposite hemisphere.



1,000 yr) for the pre- and post-transitional zones, which makes it reasonable to estimate the duration of the reversal from the thickness of the transition zone (190 m). Hence, the transition period lasted $\sim 4,500$ yr, with an uncertainty of $\sim 1,000$ yr.

The pre-transitional reversed period is characterized by an average palaeointensity of $31.5 \pm 8.5 \mu\text{T}$ ($\pm$ s.d.). This mean is $\sim 30\%$ lower than the expected Miocene field at the site, as calculated from 49 independent palaeointensity data from all over the world, obtained using the Thellier method⁷⁻⁹. We interpret this difference, significant at the 95% confidence level, as resulting from a long-term decrease in the dipole intensity before the transition (Fig. 1) support the idea of a relative decrease of the dipole with respect to the non-dipole terms before the reversal. This pre-transitional dipole decrease would have lasted at least 5,000 yr.

In contrast, the intensity of the post-transitional normal field ($46.7 \pm 20.1 \mu\text{T}$) is not significantly different from the expected average Miocene field. However, large and apparently rapid intensity fluctuations are observed during this $\sim 3,500$ -yr-long record (Fig. 2), which may reflect some instability of the newly re-established dipole.

The major feature of the Steens Mountain transition is the succession of two distinct phases (Fig. 1). The first phase is a reversed-transitional-normal flip that apparently lasted no more than $\sim 1,500$ yr because of the thickness of the recording zone. The second phase is a normal-transitional-normal 'rebound'¹ which probably lasted twice as long. The apparently brief period of time between these two phases during which normal dipole-like directions occur is marked by an increase in field intensity back to pre-transitional values (Fig. 2). Thus, we interpret these

directions as corresponding to a briefly re-established dipole configuration.

During phases 1 and 2, we found very low values of geomagnetic intensity with several minima close to $5 \mu\text{T}$. The average intensity of the typically transitional field vectors, that is, the 18 vectors directed more than 45° away from the unsigned dipole direction, is $10.9 \pm 4.9 \mu\text{T}$. This is about one-fifth of the intensity of the non-transitional Miocene field. We observe also that the r.m.s. values of the north-south, east-west and vertical components of these transitional vectors are of a comparable order of magnitude with those of the historical non-dipole field at the latitude of the site. Figure 2 shows that there is a fairly regular westward movement of the field vector as the reversal develops, which strongly suggests that at least part of the transitional field is longitudinally drifting.

Transitional geomagnetic impulses

Three large directional jumps are observed during the transition (Fig. 1), amounting to $\sim 90^\circ$ differences between successive pairs of directional groups. This is in marked contrast to most of the Steens Mountain record, where successive angular differences are more often close to $\sim 20^\circ$, suggesting a detailed sampling of the transitional field. Although we first interpreted these jumps as a result of some intermission in the eruptive process, further observations suggested that they instead reflect some large and very rapid changes (geomagnetic impulses) of the field. The most convincing evidence comes from the second impulse, which is recorded in section A from flows A46-A43 (DG 24) to A40-A38 (DG 21). Two lava flows, A42 (DG 23) and then A41 (DG 22), erupted at the beginning of this impulse. After magnetic cleaning, the top and middle of both of these flows possess

distinctly different directions of magnetization (Fig. 3). Considering the chronology of the flows and their directions of remanence, these differences are not caused by reheating by the overlying flow. Field evidence also eliminates differences resulting from volcanological processes such as filling of lava tubes or rotation of magnetized blocks during eruption.

The directions of natural remanent magnetization can sometimes be extremely scattered, even antipodal, within a flow interior because of some secondary chemical remanent magnetization developing after the cooling of the flow¹⁰. In the only such example yet reported, thermal cleaning at high temperature allows the recovery of the direction of primary remanence¹⁰. In contrast, natural remanent magnetization directions in the interior of both flows A41 and A42 are not extremely scattered before or after magnetic cleaning (Fig. 3). The directions of magnetization of the near-surface samples do not change significantly during either alternating field or thermal cleaning. Most of the blocking temperature ranges of these samples are between 500 and 550 °C, whereas the interior samples display a distribution of blocking temperatures between 20 and 600 °C. However, after destroying some secondary magnetization directed towards the present-day field, directions found at high temperatures (~500 °C) in the interior samples are not different from those obtained by alternating field cleaning, and their cleaned directions remain obviously different from those of the near-surface samples (Fig. 3). Thus, the remanence of the interior samples seems to be primary in origin, having been acquired at high temperature during flow cooling.

Therefore, we interpret the distribution of directions observed in flows A42 and A41 as resulting from a geomagnetic impulse occurring during the cooling of these flows. The near-surface directions of flows A42 and A41 yield an average inclination of 45.4°, a declination of 279.5°, with $\alpha_{95} = 3.1^\circ$. This direction differs from the interior average directions of flows A42 and A41 by $16 \pm 11^\circ$ and $34 \pm 13^\circ$, respectively (95% confidence intervals). To estimate the rate of change of the field during the impulse, we need to know the cooling history of the flows. This is only possible for flow A41, which must have acquired its magnetization before being covered by the subsequent flow which has a different magnetization direction. Assuming that the primary remanence was acquired at ~500 °C, and according to temperature measurements in Hawaiian lava lakes¹¹, we find that ~8 months should have elapsed between the time that the top and the middle of this 4-m-thick flow became magnetized. The angular rate of change would have then been $\sim 50 \pm 20^\circ \text{ yr}^{-1}$. The 95% confidence interval given here corresponds only to the uncertainties about directions, neglecting those about the cooling time, which are large but difficult to estimate. Four palaeointensity values were obtained from flows A42 and A41. Their arithmetic average is $7.1 \pm 0.9 \mu\text{T}$, indicating that this impulse occurred when the field intensity was very low. The corresponding rate of change of the field vector during the impulse would have been $6.7 \pm 2.7 \mu\text{T yr}^{-1}$.

There is similar, though qualitative, evidence that the first directional jump (Fig. 2) also corresponds to a geomagnetic impulse. The possibility that the third jump also results from an impulse cannot yet be demonstrated.

General characteristics

Comparison of sedimentary records of the Brunhes–Matuyama reversal obtained from various locations on the globe indicates that the transitional field is not dipolar^{12,13}, insofar as the reversing field is faithfully recorded in the rocks studied. To describe the transitional fields in more detail, one has to turn to volcanic rocks, which are much more reliable recorders of both the direction and intensity of the field. Indeed, only volcanic rocks allow the determination of both the 'instantaneous' field direction and absolute palaeointensity. A consideration of the new data from the Steens Mountain reversal together with other data from transitions recorded in Cenozoic volcanic sequences confirms that transitional fields are low in intensity and suggests that these fields have three other major characteristics: import-

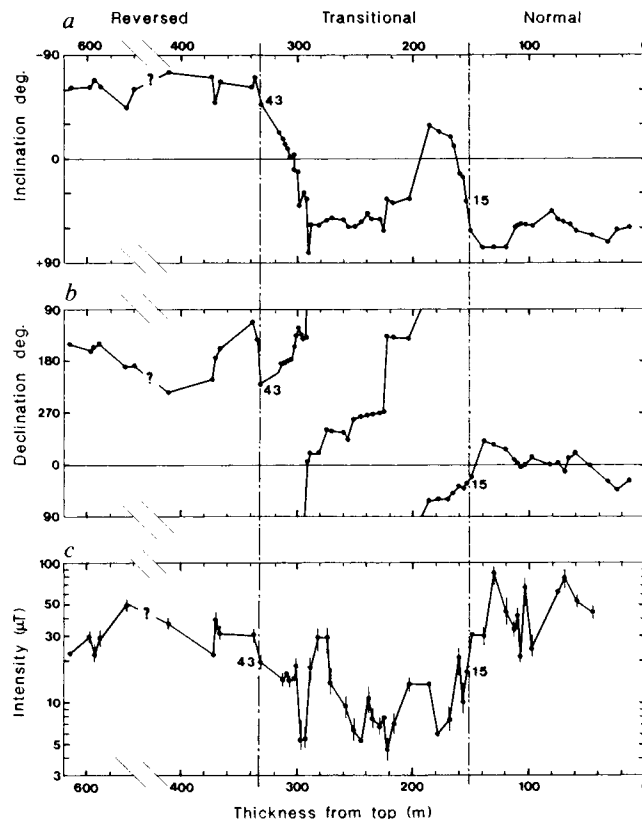


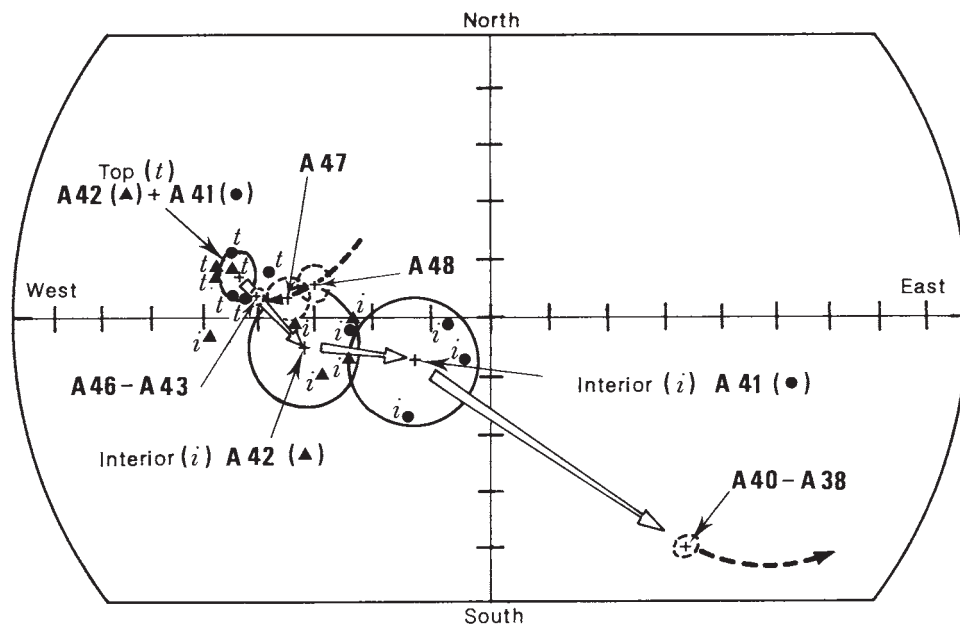
Fig. 2 The Steens Mountain vectorial record, showing average inclination, declination and intensity (logarithmic scale) of the geomagnetic field calculated for groups of successive lava flows which do not differ significantly in direction or intensity. *a, b*, 65 data points, some of which correspond to subdivisions of the directional groups within which intensity varies significantly between successive lava flows. *c*, 51 data points corresponding to the vectorial groups defined in the text. Group numbers 15 and 43 are indicated by the representative points. Average values are plotted versus thickness from the top of section A (2,950 m), so that time develops from the left to the right on the diagrams. No samples were collected between 410 and 560 m from the top. Error bars in *c* are s.e.m.; no bar indicates a single intensity determination. Uncertainties about inclination and declination are too small to be represented.

ance of non-zonal terms; occurrence of rapidly dumped oscillations; and geomagnetic impulses.

The Steens Mountain data confirm that palaeointensity of the geomagnetic field is low during reversals and suggest that a long-term dipole decrease precedes the transition. We did not find any evidence of the large intensity value thought to occur at the start of the first phase of the transition¹⁴. To what extent the average intensity of transitional fields found at Steens Mountain (~11 μT , or 20% of the non-transitional intensity) is typical is unknown because there are no comparable studies. Note that the average palaeointensity calculated from Cenozoic field reversals and excursions recorded in volcanic sequences from Iceland is only $5.5 \pm 3.1 \mu\text{T}$ (ref. 15), which suggests that average transitional palaeointensities are latitude dependent.

The importance of non-zonal components during the Steens Mountain reversal can be qualitatively understood from Fig. 1. Only sectorial or tesseral spherical harmonics can account for any deflection of the field direction from the north–south vertical plane (corresponding to pseudodeclination of 0 or 180° in Fig. 1). The second phase of the transition, which is also the longest, exhibits the directions that are the farthest from the geographical meridian. Considering the transition as a whole, the vector density of typically transitional directions (that is, >45° away from the unsigned dipole direction) is at a maximum for angular deviations between 30 and 40° from the geographical meridian (Fig. 4). Thus, most of the Steens Mountain transition is not

Fig. 3 Lambert equal-area projection of the cleaned directions of remanence of individual samples from lava flows A41 and A42, which erupted during the second geomagnetic impulse, and average directions of some pre- and post-impulse lava flows. Average directions are indicated by crosses with the corresponding α_{95} circles: solid circles, flows A41 and A42; broken circles, pre- and post-impulse lava flows. The pre-impulse directional path (broken line) is recorded successively by flows A48, A47, A46 to A43 and the tops of flows A41 and A42. Two successive records of the field direction during the impulse are provided by the interiors of flows A42 and A41. The post-impulse field direction is recorded by the overlying lava flows A40 to A38.



axisymmetrical, which is a general characteristic of Cenozoic reversals (Fig. 4). As well as the Steens Mountain reversal, data from the 71 directional groups in Fig. 4 correspond to transitions recorded by volcanic sequences in North America¹⁶⁻²⁰, Iceland^{21,22}, Japan²³ and Africa²⁴. Vector density is at a maximum for angular deviations between 40 and 70° from the geographical meridian, in marked contrast to the distribution of non-transitional field directions which would have typically displayed a maximum for a deviation of zero. The observed distribution indicates that, on average, east-west (non-zonal) components of transitional fields are at least as important as the components lying within the geographical meridian. As the latter components are a combination of zonal and non-zonal terms, this suggests that zonal and non-zonal components of transitional fields are of the same order of magnitude.

Thus, during polarity transitions, the geomagnetic field not only loses its dipolar morphology but also its axisymmetry. Similarly, the present-day non-dipole field does not show any predominance of zonal terms. The absence of a purely zonal morphology for transitional fields is compatible with the sugges-

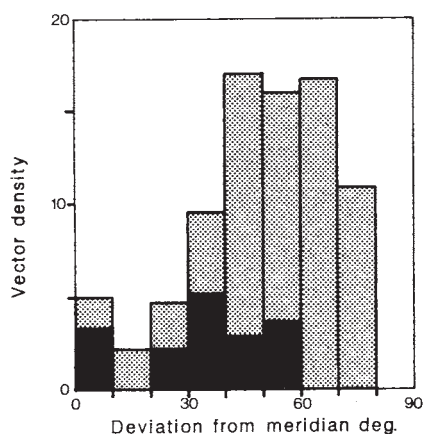


Fig. 4 Histogram showing vector density versus deviation from the geographical meridian for 71 transitional directions from Cenozoic reversals recorded by volcanic sequences (see text). All transitional directions considered are flow or directional group averages which are more than 45° away from the dipole field direction at the sampling site. Vector density is calculated using one as the value of the area on the sphere corresponding to the 40–50° range in deviation from the meridian. Black columns, data from the Steens Mountain transition.

tion that the frozen-flux approximation is valid during reversals²⁵. However, this agreement does not imply that flux diffusion is negligible over the full time of a reversal. In particular, the beginning of the Steens Mountain reversal, which seems to be only moderately non-zonal (Fig. 1), could result from ohmic decay within a geodynamo temporarily inoperative as a consequence of the axial symmetry²⁶.

The notion that the transitional state of the geodynamo corresponds to an oscillating field was proposed by Braginsky²⁷. In contrast, more recent models suppose that the field reverses in a progressive way, through either some spreading of the reversal over the core surface^{13,28} or by reversible energy transfer from the dipole to non-dipole terms²⁹. The entire directional path observed during the Steens Mountain transition can be interpreted as corresponding to a rapidly damped oscillation of the field. Although other records of reversals by volcanic rocks are not as detailed as the Steens Mountain record, examples have been reported in which the field reverses polarity and then comes back to its initial polarity before reversing again, even in such incomplete records^{19,30}. Such observations favour models of the geodynamo that allow a highly damped oscillatory state of the geomagnetic field during reversals.

The geomagnetic impulses occurring during the Steens Mountain transition can be considered as impulses of the first order time-derivative of the field. They may be interpreted as large and possibly global changes of the field configuration with time constants of the order of 1–2 yr. By comparison, we note that the same order of magnitude has been proposed recently for the smoothing time of the 1969 impulse resulting from filtering by the mantle³¹. The rates of change of the field components during the Steens Mountain transitional impulses would have been very large. For the second impulse, the rates of change of the three components would have been between 15 and 50 times larger than the maximum rates calculated for the historical non-dipole field³².

Obviously diffusion of field lines cannot explain such rapid changes; even for harmonic degrees as large as 10, the decay time is still equal to a few hundred years³³. Larger-degree harmonics with decay times of the order of a few years require implausibly large fields at the core-mantle boundary. Under the frozen-flux approximation, however, the transitional geomagnetic impulses can be accounted for by a large increase in fluid velocity at the core surface. This suggests an increase in the level of turbulence within the liquid core during reversals. This increase in kinetic energy can be of thermal origin, resulting from too steep a thermal gradient within the core for a convective

regime to be preserved³⁴. Alternatively, this increase can result from a decrease in magnetic energy. We have shown that, on average, the intensity of the geomagnetic field at the Earth's surface reduces to one-fifth of its normal value during the Steens

Mountain polarity transition. If the field intensity within the core also decreases during reversals, the liquid can become more unstable as part of the magnetic energy is transferred into kinetic energy³⁵.

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Primary structure and transmembrane orientation of the murine anion exchange protein

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The amino-acid sequence of murine band 3, deduced from the nucleotide sequence of a complementary DNA clone, confirms that this integral membrane glycoprotein is composed of two major structural domains which correlate with its dual functions as the anchor for the erythrocyte cytoskeleton and as a plasma membrane anion antiporter. This latter activity resides within a highly hydrophobic domain that crosses the plasma membrane at least 12 times.

BAND 3 is the major integral membrane glycoprotein of the mammalian erythrocyte¹. It functions as an anion antiporter, mediating the one-for-one, obligatory and electroneutral exchange of chloride and bicarbonate across the plasma membrane (see refs 2-6 for review). This rapid process ($t_{1/2}$ = 40-60 ms), catalysed by the ~1,000,000 molecules of band 3 per erythrocyte³, increases the capacity of the blood to transport CO₂ from the respiring tissues to the lungs. Through its tight association with ankyrin^{7,8}, band 3 anchors the mesh-like cytoskeletal network of spectrin and ankyrin to the inner surface of the erythrocyte plasma membrane (see ref. 9 for review).

As a consequence of its physiological importance and relative abundance, band 3 structure and membrane topology have been characterized extensively by biochemical techniques including proteolytic mapping, inhibitor binding and covalent modification (see ref. 4 for review). Band 3, 929 amino acids long, has two distinct structural domains, one containing the anion exchange activity and the other involved in cytoskeletal interactions.

The ~400 N-terminal amino acids of band 3 are hydrophilic and face the cytoplasm. This negatively charged domain possesses the high-affinity binding sites for ankyrin⁸ as well as, in human band 3, haemoglobin¹⁰ and several glycolytic enzymes^{11,12}. Removal of the cytoplasmic domain of band 3 by proteolytic cleavage of erythrocyte ghosts results in no loss of anion transport activity¹³, demonstrating that this function resides entirely within the C-terminal ~500 amino acids of the protein. Studies on the topology of this domain suggest that it traverses the membrane at least seven times^{14,15}; hitherto it has

only been possible to obtain the sequence of small fragments of this region in the human protein.

Here we report the isolation and the sequence of cDNA clones encoding the full length of murine band 3 messenger RNA. This is the first plasma-membrane anion transporter to be sequenced. Previous studies have established that human and murine band 3 have very similar sizes and biochemical properties¹⁶. We propose a model for band 3 topology that incorporates data from many biochemical and physiological studies.

Isolation of cDNA clones

In mice, anaemic stress induces proliferation in the spleen of erythroid precursor cells that actively synthesize erythrocyte proteins^{17,18}. Polyadenylated RNA from these spleens was used to construct a cDNA library in the expression vector λ gt11 (ref. 19). We screened this library with a polyclonal antibody²⁰ against purified mouse erythrocyte band 3, initially revealing several positive clones, the inserts from which hybridized to each other and to a 4,300-nucleotide mRNA in total spleen RNA (data not shown). The longest of these, pB33, contains a 1,800-base-pair (bp) insert, which was shown subsequently to encompass only ~280 bp of the C-terminal protein coding region, but which extends throughout the entire 3' untranslated region (1,500 bp). We used pB33 as a hybridization probe to screen a second cDNA library, constructed to optimize the yield of full-length transcripts (see Fig. 1 legend). Sequencing the longest insert (clone pB399; 2.7 kilobases (kb)), revealed that it did not contain the N-terminus of native band 3, judged by the homology of the deduced amino-acid sequence with that of the N-terminus

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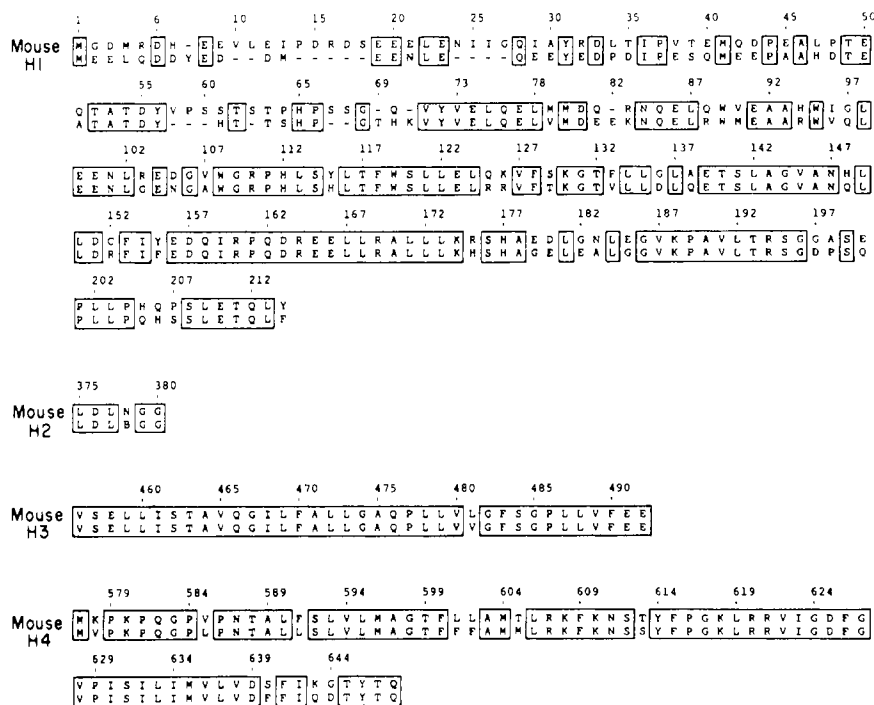


Fig. 2 Alignment of sequences of fragments of human band 3 with the deduced sequence of the mouse protein. The ALIGN program of Dayhoff *et al.*⁵² was used to obtain the optimal alignment using the mutation data matrix⁵². Shown are the alignments for an N-terminal fragment, H1 (ref. 21), a short peptide from the N-terminus of a M_r 14,000 chymotryptic fragment, H2 (ref. 25), and two fragments from the membrane-associated domain, H3 (ref. 25) and H4 (ref. 26). Boxes indicate amino acid identity between the two species. The alignment scores were compared with those obtained from 150 randomizations of the entire mouse sequence, using a break penalty of 2. The scores for the alignments were 14.6 (H1), 2.66 (H2), 15.5 (H3) and 12.2 (H4) standard deviations, respectively.

of human erythrocyte band 3 (ref. 21; see below). Therefore, we re-screened the library using a 5' fragment of pB399 as a probe, and isolated a cDNA clone, pB3SP4, encompassing 127 bp of 5' untranslated region as well as the entire 2,900-bp open reading frame encoding murine band 3.

Band 3 sequence homology

The complete sequence of 4,383 nucleotides compiled from the cDNA clones pB33, pB399 and pB3SP4 is shown in Fig. 1. The size of the full-length cDNA represented by the three clones agrees well with that of the mRNA (data not shown). The overlapping regions of the three clones have identical sequences. A single open reading frame extends for 929 codons from the ATG at nucleotide 1 to the TGA at base 2,919. There are two in-frame ATG codons at the beginning of the open reading frame (residues 1 and 4). We have assigned the first Met codon as the initiator because it is the first in-frame ATG downstream of the (in phase) stop codon at base -63. The sequences flanking this ATG, but not the other one, are homologous to the highly conserved sequence that flanks functional initiation sites in eukaryotic mRNAs: AXX AUG G²². The relative molecular mass (M_r) of the predicted polypeptide is 103,000, in good agreement with estimates of 90,000–100,000 obtained from SDS-polyacrylamide electrophoresis of the native human¹ and murine²³ proteins. The predicted C-terminal amino acid of the murine sequence is valine, which is also the C-terminal residue of the human protein²⁴. Indeed, of the 12 C-terminal residues predicted from the mouse cDNA sequence, 11 are identical with those determined by carboxypeptidase-Y digestion of human band 3 (R. Reithmeier, personal communication). Parts of the the amino-acid sequence deduced from the cDNA sequence contain high homology to that of the several known fragments of human erythrocyte band 3 (Fig. 2), proving that the clones we have isolated encode the murine erythrocyte anion exchange protein.

The most striking homology is between residues 455 and 492 of murine band 3 and the 38-amino-acid fragment from the human protein designated H3 (ref. 25) in Fig. 2. There is only a single conservative (Leu → Val) substitution between the two sequences; there are no insertions or deletions. Excellent alignment is also observed between the deduced sequence of murine band 3 and the sequence of a peptic fragment (P5) of the human protein designated H4 (ref. 26; Fig. 2); only 10 of the 72 residues differ and alignment of the sequences required no insertions or

deletions. The sequence of the N-terminal 201 residues of human band 3 (ref. 21), designated H1, exhibits a slightly lower homology with the sequence deduced from the murine band 3 clone.

Structural domains of band 3

A hydropathy plot²⁷ (Fig. 3) reveals that murine band 3 can be roughly divided into three domains. The N-terminal 420 residues constitute the hydrophilic cytoplasmic domain with a negative net charge (65 acidic and 37 basic residues). The central 450 amino acids are predominantly amphipathic, and are intimately associated with plasma membrane lipid. In this domain, groups of hydrophobic residues, which correspond to the peaks *a*–*j* in Fig. 3, are interspersed with polar, predominantly basic, residues, depicted schematically in Fig. 4. The extreme 32 C-terminal residues of band 3 may constitute a third domain. Eleven of these are either Glu or Asp, making it unlikely that the C-terminus is buried within the lipid bilayer.

Cytoplasmic domain

The binding of haemoglobin¹⁰ and glycolytic enzymes^{11,12} to the cytoplasmic domain of human band 3 is mediated by the first 11 residues of the band 3 polypeptide²⁸. The apparent absence of glyceraldehyde 3-phosphate dehydrogenase from mouse erythrocyte ghost preparations (V. Patel, personal communication) is consistent with the lack of homology between the mouse and human sequences in this N-terminal undecapeptide.

Other features of the cytoplasmic domain are more conserved: both human²⁹ and murine band 3 possess six Cys residues (Fig. 4). Three of the murine band 3 cysteines are in the cytoplasmic domain (CH65), consistent with studies on human band 3 (ref. 29).

Low *et al.*³⁰ propose that the cytoplasmic domain of CH65 is a homodimer with a regulated hinge rich in proline residues. These prolines apparently disrupt the α -helical structure of the polypeptide, rendering it susceptible to proteolysis. Note that these prolines (at positions 161, 188, 201 and 204) are all conserved between human and murine band 3 (compare with Fig. 2); Fig. 4 shows them to be clustered around a trypsin cleavage site at position 194. (Interestingly, two other proline-rich regions in the predicted mouse sequence are found at the known primary sites of intracellular and extracellular proteolysis, between residues 361–368 and 567–586, respectively.)

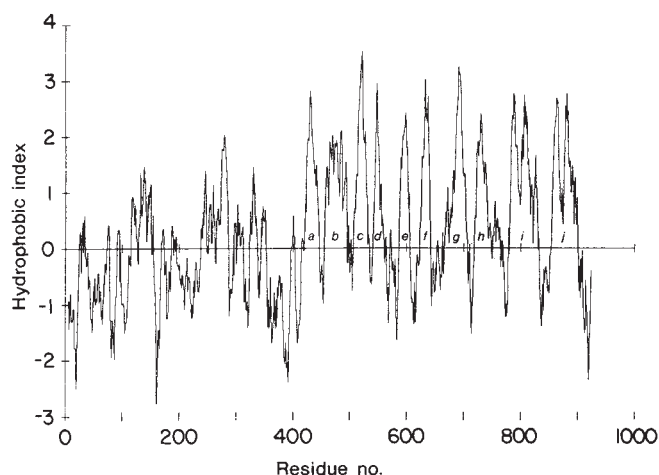


Fig. 3 Hydropathy plot of the deduced amino-acid sequence of murine band 3 using the algorithm and hydropathy values of Kyte and Doolittle²⁷. Hydrophobicities were averaged over windows of 11 amino acids. The 10 hydrophobic regions are designated a-j.

Between the proposed hinge and a region rich in tryptophan (residues 89–119), also highly conserved, is the purported antigenic region of band 3 (ref. 30). Monospecific polyclonal antibodies against total human band 3 recognize determinants located within a M_r 20,000 fragment of the cytoplasmic domain³¹; this site is sufficiently distant from the N-terminus so that the binding of antibody does not interfere with the binding of haemoglobin³⁰. The region between residues 125–138 contains only 57% homology between human and mouse, whereas the residues flanking it on either side are 90–95% homologous, consistent with the lack of cross-reactivity of anti-human band 3 antisera with the mouse protein and vice-versa²³.

The ankyrin binding site on band 3 has not been identified; Low *et al.* tentatively place it between the hinge region and the immunoglobulin G region, because antibodies against band 3 compete with labelled ankyrin for binding³⁰. The high-affinity ankyrin-binding site is probably conserved among different

species and, perhaps, among different non-erythroid ankyrin-binding proteins.

Membrane-associated domain

The C-terminal half of band 3 contains 10 long hydrophobic stretches (Fig. 3a–j). Regions with hydropathy averages >1.5 are usually tightly associated with lipid, and, in the case of several proteins, span the membrane in an α -helical configuration^{27,32}. Our two-dimensional model indicates that seven of these peaks correspond to sequences of amino acids that span the phospholipid bilayer once (Fig. 3a, c–h) and that two (b, i), and possibly a third (j), span twice.

Peaks a, c, e, g and h, designated single-spanning regions 1, 4, 6, 8 and 9, respectively, correspond to continuous stretches of 20–24 extremely hydrophobic amino-acid residues and fit the criteria for forming membrane-traversing α -helices^{27,32}.

Chymotryptic digestion of intact erythrocytes cleaves band 3 into a carboxy-terminal glycosylated peptide, CH35, and an amino-terminal non-glycosylated CH65 (Fig. 4). Sequence data on the N-terminus of human CH35 (ref. 26) positions this site between the putative membrane-spanning segments 5 and 6, thus this segment must be extracytoplasmic. The extensive homology between the amino-acid sequences of murine and human band 3 in the peptic fragment studied by Brock *et al.*²⁶ allows us to assign the topological asymmetry to the corresponding membrane-spanning segments 5–8 of the mouse sequence. Of the two potential sites (Asn-X-Ser/Thr) for attachment of the single N-linked oligosaccharide, the one at residue 611 in the murine sequence corresponds to the site in the human protein²⁶ shown not to contain carbohydrate. Therefore, the site at Asn 660 must be the extracellular attachment site, consistent with the finding that the human Tyr 646 is a substrate for extracellular radioiodination²⁶.

The region encompassing segments 1–5 corresponds to part of the M_r 17,000–22,000 fragment, CH17, generated by chymotryptic digestion of ghosts. It must span the membrane an odd number of times as it results from both an intra- and an extracellular proteolytic cleavage³³. Studies with surface-specific radiolabelling reagents demonstrate that it traverses the bilayer at least three times¹⁵.

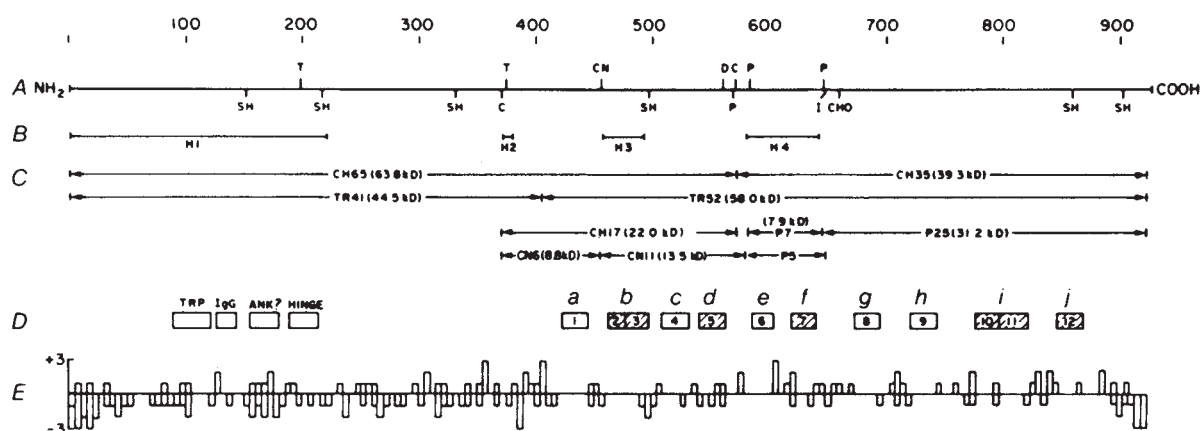


Fig. 4 Domain map of band 3. **A**, The scale is the complete amino-acid sequence of murine band 3 deduced from the nucleotide sequence. Probable sites for proteolytic cleavage by trypsin (T) at position 194 (R. Reithmeier, personal communication) and 381 (refs 15, 25), chymotrypsin (C)³³, cyanogen bromide (CN)^{15,25} and papain (P)¹⁴ are indicated. Cysteine residues (SH), binding site for H₂DIDs (D), and the known site for exofacial radioiodination (I)²⁶ are all designated. The proposed site for attachment of the N-linked oligosaccharide (CHO) is shown. **B**, Alignment of the fragments from human band 3. The codes H1–H4 are defined in Fig. 2 legend. **C**, Primary fragments arising from digestion of human band 3 with extracellular chymotrypsin, CH65 and CH35^{13,24}; intracellular trypsin, TR41 and TR52³³; extracellular papain, P7 and P25¹⁴; extracellular pepsin, P5^{26,53}; and cyanogen bromide cleavage of CH17, CN6 and CN11^{15,25}. Numbers in parentheses are the actual M_r values of the fragments calculated from their amino-acid composition. These values do not take into account the contribution of the single N-linked oligosaccharide. **D**, Structural features within the N-terminal cytoplasmic domain are: tryptophan-rich (TRP)³⁰, antibody-binding (IgG)^{30,31}, ankyrin-binding (ANK) and hinge are indicated (see text). Locations of the hydrophobic peaks from the hydropathy plot (Fig. 3) are indicated by the letters a–j and the corresponding presumed membrane-spanning regions are designated by the numbers 1–12. Hatched boxes indicate that the region is likely to be amphipathic (see text). **E**, Charge plot. Location of charged residues in the mouse band 3 sequence is indicated by the bar graph, the positions corresponding to the sequence numbers at the top of the figure. The basic residues (Arg and Lys) are assigned a value of +1 while the acidic residues (Glu and Asp) are assigned a value of –1. The bars represent the total (+) or (–) values, above and below the horizontal axis, respectively, over 5 residues.

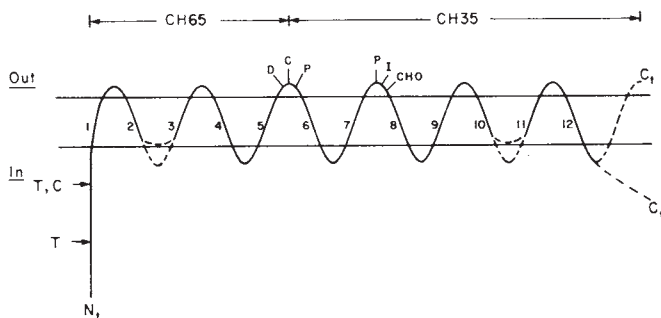


Fig. 5 Model for band 3 orientation in the plasma membrane. A two-dimensional projection of the orientation of the cytoplasmic domain and the membrane-spanning regions in the lipid bilayer is shown. The sites of oligosaccharide attachment (CHO), H₂DIDS binding, as well as the major extracellular sites of proteolysis, are indicated. Abbreviations are as described in Fig. 4 legend.

If we assume that peaks *a*, *c* and *d* of the hydrophobicity plot correspond to three membrane-spanning segments, 1, 4 and 5, then either region *b* (residues 453–491) does not span the membrane, or it must do so twice. Hydrophobicity peak *b* is quite broad, encompassing 38 moderately apolar residues. These residues can be arranged to form a structure composed of two antiparallel amphipathic helices with Pro 477 forming a turn within the membrane or just at the interface between the bilayer and the cytoplasm. Analysis of the helical amphiphilicity³⁴ suggests that both helices formed by this region of the protein would have all their polar residues projecting from a single face of each helix. Thus, we conclude tentatively that CH17 possesses five membrane-spanning α -helices, of which three (2, 3 and 5) are amphipathic. Helices 1 and 2 could form a hairpin that functions as the internal uncleaved signal sequence²⁰ for insertion of the nascent chain into the endoplasmic reticulum membrane.

Labelling of intact human erythrocytes with a reagent to which the membrane is impermeable has led to the identification of three exofacial lysines in CH17: one on the cyanogen bromide fragment CN6 and two on CN11 (see Fig. 4 and ref. 15). One of the lysines in CN11 binds extracellular 4,4'-diisothiocyanato-2,2'-dihydrostilbene disulphonate (H₂DIDS), a potent inhibitor of anion transport³⁵. Our topological model (Fig. 5) is consistent with these labelling studies, predicting that all three lysines in mouse CH17 (positions 449, 558 and 561) are exofacial. These

data also allow us to assign the site for H₂DIDS binding to either Lys 558 or Lys 561.

Virtually nothing is known about the transmembrane orientation of the C-terminus of band 3 corresponding to hydrophobic peaks *g*–*j*. Our model proposes four or five membrane crossings. The region corresponding to the 43 residues of peak *i* in Fig. 3 resembles membrane spans 2 and 3 in its ability to form an α -helical hairpin in which Arg 800 is situated just at the cytoplasmic surface and the C-terminal 24 amino acids return to the extracellular surface. The proline residues at positions 796 and 802 may be involved in forming the hairpin structure. These two spanning segments, along with segment 12, could form moderately amphipathic helices. Following an extremely polar stretch between residues 832 and 850, there is a short region of 18 mixed hydrophobic and polar residues that forms the N-terminal part of peak *j* in Fig. 3. This is the longest stretch of apolar amino acids between position 850 and the C-terminus of the protein. We propose that this segment spans the membrane once, as an amphipathic α -helix (Fig. 5, segment 12), positioning the C-terminus of the protein inside the cell. Alternatively, the protein may traverse the membrane once more, leaving the C-terminus outside the cell. These two possibilities are shown as dotted lines in the model (Fig. 5).

Analysis of the predicted sequence of murine band 3 reveals that the protein possesses 12 membrane-spanning regions. Five of these are hydrophobic and probably traverse the bilayer as single-spanning α -helices. The seven remaining membrane-spanning regions contain both hydrophobic and polar residues that cross the plasma membrane as amphipathic helical structures, confining their polar and charged side chains to a single face of the helix. These helices, oriented with their axes transverse to the plane of the membrane, could cluster, forming at least one hydrophilic region within the bilayer across which the charged residues involved in the anion-exchange process may pair. Clustering of amphipathic helices round an aqueous 'pore' has been proposed for other transport proteins which span the membrane several times^{32,36}. Significantly, either of the two lysine residues (558 or 561) which bind to stilbene disulphonate inhibitors of anion exchange are associated with such an amphipathic region.

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Is GX5-1 a millisecond pulsar?

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The bright galactic bulge source GX5-1 (4U1758-250) has recently been observed¹ to exhibit quasi-periods between 25 and 50 ms, with a correlation between the corresponding frequencies f and the count rate I given by $d \log f / d \log I \sim 2.5$. This is the first source observed to exhibit such a phenomenon. We propose here that this behaviour results from fluctuations in accretion rate onto a millisecond pulsar, which has been spun up by accretion to its steady-state spin rate. According to models that use spin-up by accretion to bring the neutron stars to steady-state fast short periods, galactic bulge sources are likely progenitors of millisecond pulsars²⁻¹⁰. After steady state is achieved in these systems, accretion may continue without further spin-up, so that for a substantial fraction of the lifetime of a galactic bulge X-ray source, the neutron star may already be spinning rapidly at the equilibrium spin. The behaviour of GX5-1 can be explained in terms of deviations from this equilibrium state resulting from changes in the accretion rate $\dot{M}$; GX5-1 may, therefore, be a new millisecond pulsar.

For a neutron star of mass M with a dipole magnetic field B_8 (in 10^8 G), radius R_6 (in 10^6 cm) and mass accretion rate $\dot{M}_{17}$ (in 10^{17} g s⁻¹), the Alfvén radius r_A (inner edge of the keplerian accretion disk) is given by

$$r_A = 1.1 \times 10^6 B_8^{4/7} R_6^{12/7} \dot{M}_{17}^{-2/7} (M/M_\odot)^{-1/7} \text{ cm} \quad (1)$$

We have used the normalization of ref. 2 in this equation. The keplerian angular velocity at r_A is

$$\Omega_K(r_A) = (GM)^{1/2} r_A^{-3/2} \\ = 9.5 \times 10^3 B_8^{-6/7} R_6^{-18/7} \dot{M}_{17}^{3/7} (M/M_\odot)^{5/7} \text{ rad s}^{-1} \quad (2)$$

where G is the gravitational constant. Let us denote the equilibrium values of $\dot{M}$ and r_A by $\dot{M}_0$ and r_A^0 . These values determine the equilibrium rotation rate of the star. If $\dot{M}$ deviates slowly from $\dot{M}_0$, the star accretes from the corresponding r_A of equation (1). The specific angular momentum of the accreted matter also varies with this 'instantaneous' r_A ; however, the star's rotation rate will not be affected by such torque variations on time scales much shorter than the spin-up time scale, $\geq 10^7$ yr. Our basic claim is that if the accretion process is controlled by the interaction (matching) between the magnetosphere co-rotating with the star and matter at the instantaneous Alfvén radius, then the luminosity will display a 'beat' frequency¹¹

$$\omega = \Omega_K(r_A) - \Omega_0 \quad (3)$$

in the observer's frame, where Ω_0 is the stellar steady-state angular frequency. The nature of this matching is discussed below.

If the observed X-ray flux originates entirely at the surface of the neutron star, its time dependence is simply that of $\dot{M}$, through $L = GMM/R$. The luminosity L is related to the observed count rate I by

$$L = 8.1 \times 10^{37} \text{ erg s}^{-1} \left(\frac{d}{10 \text{ kpc}} \right)^2 I(1,000 \text{ c.p.s.}) \quad (4)$$

where d is the distance to GX 5-1. Using equations (2), (3) and (4), we get

$$\omega = a I^{3/7} - \Omega_0 \equiv a(I^{3/7} - I_0^{3/7}) \quad (5)$$

Table 1 Estimated parameters for the neutron star

P_{obs} (ms)	I (1,000 c.p.s.)	r_A (10^6 cm)	$P_K(r_A)$ (ms)	$\Omega_K(r_A)$ (rad s ⁻¹)
25	3.1	4.5	5.20	1,200
50	2.4	4.84	5.84	1,080
	B (10^9 G)	r_A^0 (10^6 cm)	P_0 (ms)	Ω_0 (rad s ⁻¹)
	5.5	5.26	6.6	950

Alfvén radii and keplerian rotation rates corresponding to the observed quasi-periods 25 ms and 50 ms and the rotation period P_0 and magnetic field B are given. These values are obtained from equation (5), with $M = 1 M_\odot$ and $\dot{M}_{17} = 1$.

where I is the count rate in 1,000 c.p.s. and a is given by $a = 2.3 \times 10^4 B_8^{-6/7} R_6^{-15/7} (M/M_\odot)^{2/7} (d/10 \text{ kpc})^{6/7}$. From the observed values of $P = 50$ ms at $I = 2,400$ c.p.s. and $P = 25$ ms at $I = 3,100$ c.p.s., we obtain $\Omega_0 = 958 \text{ rad s}^{-1}$, corresponding to a rotation period $P_0 = 6.6$ ms for the neutron star, and $a \approx 745$, which gives $B \approx 5.5 \times 10^9$ G (see Table 1). These are, indeed, typical values for millisecond pulsars^{2,6} spun up by accretion, particularly for those born in bright galactic bulge sources⁶. Note that if the observed frequency is actually the n th harmonic of the fundamental beat frequency²⁰ then the inferred Ω_0 values would be n times smaller, r_A values $n^{2/3}$ times larger and B fields $n^{7/6}$ times larger than the values given in Table 1.

From equation (5), we further find that

$$\alpha \equiv \frac{d \log f}{d \log I} = \frac{d \log \omega}{d \log I} = \frac{3}{7} \frac{1}{1 - \Omega_0 / a I^{3/7}} \quad (6)$$

α has the values 2.06 at $I = 3,100$ c.p.s. and 3.70 at $I = 2,400$ c.p.s. The best fit with a constant $\bar{\alpha} \equiv d \log \omega / d \log I$ to the $\omega(I)$ curve, given by equation (5), yields $\bar{\alpha} = 2.68$, in very good agreement with the reported value of ~ 2.5 . Indeed, this is true for any model with a beat frequency given by $\omega = a I^x - \Omega_0$ for $0 \leq x \leq 2.7$. Fitting such models in the observed range $2,400 \text{ c.p.s.} \leq I \leq 3,100 \text{ c.p.s.}$, one obtains, for each x , a range of $\alpha(I)$ between ~ 2 and ~ 4 ; this range narrows with increasing x , reaching the single value $\alpha = \bar{\alpha} = 2.7$ at $x = 2.7$, which is the limiting case where $\Omega_0 = 0$ (the neutron star is not rotating).

It is encouraging to find that a whole class of 'kinematic' models that take the observed quasi-period to be beat frequencies, with any rotation rate for the neutron star, can fit the observed α , provided that Ω_K increases with increasing $\dot{M}$. Furthermore, the particular dependence of r_A and of Ω_K on $\dot{M}$, given in equations (1) and (2), or something close to that, must hold very generally¹², as should the relation $L \sim GMM/R$. If, for example, the observed X rays originated at the instantaneous Alfvén radius, we would have $L \sim GMM/r_A$. This gives an exponent $x = 1/3$, instead of $3/7$, in equation (5), which then yields $\Omega_0 = 1,285 \text{ rad s}^{-1}$, $P_0 \approx 4.9$ ms and $B \approx 3.6 \times 10^9$ G, values not very different from those obtained with $x = 3/7$. Indeed, all models with $0 \leq x \leq 0.6$ give equilibrium rotation periods $P_0 < 10$ ms for the neutron star. Note that the kinematic model requires $\Omega_K(r_A) > \Omega_0 \equiv \Omega_K(r_A^0)$; hence, the instantaneous Alfvén radii r_A must have been closer to the star than r_A^0 , and $\dot{M}$ must have been larger than $\dot{M}_0$ throughout the observations of the quasi-periodicities in GX 5-1; otherwise, the beat frequency would have been $\omega = \Omega_0 - \Omega_K(r_A^0)$, and negative values of the index α would have been observed.

As an example of a type of physical mechanism that would result in such beat frequencies, we propose a 'magnetic gate' scenario operating through magnetic matching between a field configuration tied to the matter rotating at keplerian velocity at the instantaneous inner boundary of the disk, r_A , and the field configuration co-rotating with the neutron star. We start from an analogy with recent findings concerning the geomagnetosphere and the solar wind. Empirical correlations between magnetic storm and substorm activity in the Earth's magnetosphere

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and the energy flux from the solar wind entering the magnetosphere were established by Akasofu and Perreault¹³⁻¹⁵. They find that, among many observable parameters of the solar wind, the quantity that correlates with the rate of energy dissipation $u(t)$ in magnetospheric storms is the energy flux into the magnetosphere from the solar wind, $\varepsilon(t)$,

$$\varepsilon(t) = VB^2 L_0^2 \sin^4 \frac{\theta}{2} \quad (7)$$

where V is the wind speed, B is the solar wind magnetic field magnitude at the front of the terrestrial magnetosphere, located at radius L_0 , and θ is the local angle between the solar wind magnetic field and the Earth's magnetic field at that boundary. In equation (7), $L_0^2 \sin^4 \theta/2$ is the effective area through which the external flux enters the magnetosphere. Thus, the flux entering the Earth's magnetosphere is maximum at locations on the boundary where $\theta = \pi$ (ref. 16), where the rate of field line reconnection is presumably maximum. Moreover, the time dependence of this behaviour is not smeared by motions of the plasma. The material flow will thus be enhanced at $\theta = \pi$, and the flow pattern will be modulated by the instantaneous distribution of θ . In particular, if the two flows are simple rotations at different rates, the pattern of relative angles θ will rotate at their frequency difference, and the energy dissipation will be modulated accordingly.

To apply these ideas to the accretion disk, we first note that the energy flux arriving at the boundary is determined by the mass transfer through the keplerian disk. Close to r_A (within a thin boundary region^{17,18}), disk matter is close to pressure balance with the magnetospheric field. Taking $V \equiv V_K(r_A) \equiv V_A$, $B^2 \equiv 4\pi\rho V_A^2$, $L_0 = r_A$ and $4\pi r_A^2 \beta \rho V_A = \dot{M}$, where β is a dimensionless parameter reflecting the geometry of accretion across the Alfvén surface and ρ is the density of the matter, equation (7) gives

$$L(r_A(t)) = \frac{\sin^4 \frac{\theta}{2}(t)}{\beta} \frac{G\dot{M}(t)}{r_A(t)} \quad (8)$$

An average of this quantity over the boundary will determine the influx of matter into the magnetosphere. Thus the observed luminosity will contain some modulation at the beat frequency due to the time dependence in equation (8), through

$$\sin^4 \frac{\theta}{2} = \frac{3}{8} - \frac{1}{2} \cos \theta + \frac{1}{8} \cos 2\theta$$

We now postulate (see ref. 20): (1) that there is some well-defined magnetic field pattern at the boundary r_A^0 ; (2) that this pattern is fixed with respect to the matter rotating at $\Omega_K(r_A^0)$; (3) that it has some finite width Δr around r_A^0 , which we shall call the coherence length. For a non-aligned rotator, θ will generally depend on the azimuthal angle ϕ about the rotation axis of the star and the disk (assumed to coincide), and the only time dependence is rotation at $\Omega_K(r_A^0) = \Omega_0$. As $\dot{M}$ increases and r_A moves inward, pieces of the field pattern move in with the matter and adopt the new keplerian rotation rate $\Omega_K(r_A)$, without substantial smearing. We now have a pattern of θ which is no longer co-rotating with the magnetosphere: the pattern is now rotating at the beat frequency $\Omega_K(r_A) - \Omega_0$ with respect to the magnetospheric field. Hence, a modulation in accretion rate at the beat frequency (or some multiple of it) will appear. This state will be clearly distinct from equilibrium (at r_A^0) once r_A has moved in from r_A^0 by a few Δr . Furthermore, there should also be another memory effect—as r_A moves even further in, the field distribution carried by the matter should lose its coherence altogether.

The above picture can also explain the observed behaviour of the amplitude of the quasi-periodicity. At the lowest frequency observed (that is, when r_A is closest to $r_A^0 > r_A$), 8% of the received flux is quasi-periodic; however, the quasi-periodic fraction decreases with frequency until, at the highest observed frequency (r_A furthest from r_A^0), the quasi-periodic behaviour disappears altogether. We may estimate Δr from the observed

full width at half maximum (FWHM) in the power spectra displaying the quasi-periodicity: $\Delta\omega/\omega \sim 25\%$ gives $\Delta r/r_A^0 \approx 2/3\Delta\omega/\omega[1-(r_A/r_A^0)^{3/2}](r_A/r_A^0) \approx (2-3\%)$. This is consistent with our estimates of the maximum and minimum Alfvén radii involved: $(r_A^0 - r_A^{\max})/r_A^0 \approx 8\%$ and $(r_A^0 - r_A^{\min})/r_A^0 \approx 14\%$ (see Table 1). A coherence time scale for the field pattern to follow radial adjustments to r_A can also be defined as $\tau \equiv \Delta r/V_r$, where V_r is the radial velocity of matter through the disk. This time scale τ must clearly be small compared with the time scale of $\dot{M}$ variations. τ is a measure of the decay time (in the radial direction) of the magnetic field pattern. Thus, if our interpretation of the observation is correct, the observed FWHM $\Delta\omega$ provides a clue to this decay time. Note that it is the instantaneous value of $\dot{M}$ at r_A which gives the correlation; as matter falls in from there rather rapidly, no delay in the correlation is expected.

It is not clear whether the present observations reflect an episode of increasing $\dot{M}$ alone or whether, in the case of decreasing $\dot{M}$ (r_A increasing from r_A^0), the field pattern does not survive coherently. We emphasize that decreasing $\dot{M}$ would give negative values of α , so that observations of negative α would indicate variations in both directions from the equilibrium configuration at r_A^0 . Note that GX5-1 may still be, on the average, spinning up.

One particularly attractive model for realizing the topological discontinuities needed for having the beat-frequency effect is, simply, accretion of magnetized clumps of matter²⁰. As each clump moves across the co-rotation ring at r_A^0 to r_A , it is absorbed in the magnetosphere at the beat rate; absorption of N incoherent clumps only reduces the modulation by $\sqrt{N}$. A logical consequence from such model is red frequency noise in the accretion²⁰. Note, that obscuration and inobservability of the fundamental stellar pulsations at Ω_0 may be here simply due to a thick disk²⁰.

The problems of magnetosphere accretion disk interaction have been discussed extensively (see, for example, refs 12, 17, 18, 19 and references therein). Solutions to particular cases and the difficulties and special assumptions involved testify to the complexity of the problem. Examples of other physical interaction to make the beat frequency observable are discussed in ref. 20 (M.A.A., F. K. Lamb, J.S. and N. Shibazaki, in preparation). On the observational side, further observations of GX5-1 and other galactic bulge sources are of great interest. An observation of a rotation period in the millisecond range as predicted would provide strong support for our kinematic model, but may be elusive in view of the absence so far of any detection of a rotation period and in view of the theoretical picture of galactic bulge sources²¹. One set of past observations, those of the 1,000-s quasi-periodicity in the 8-s pulsating source 4U1626-67²², as well as the 0.5-s quasi-periodicity in the rapid burster²³ and the quasi-periodicity in SCO X-1 (J. Middleditch and W. Priedhorsky, personal communication) may be possible candidates for similar beat frequency effects.

The particular mechanism described above for modulations at the beat frequency may not be unique. A search for similar effects in slow rotators and in massive X-ray sources may give further clues to the differences in the accretion process between these different classes of X-ray sources and will assist in sorting out other possible physical models.

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Onset of sublimation in comet P/Halley (1982i)

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We report here the first direct evidence for the onset of sublimation of a comet nucleus. Emission due to CN observed in spectra of comet P/Halley (1982i) provides evidence for the development of the gas coma. Broad-band photometric observations of the comet indicate that the dust coma developed near a pre-perihelion heliocentric distance, $r \sim 6$ AU. We have derived rates of gas production and brightening for comet P/Halley at $r \sim 4$ –6 AU and use the mean pre-perihelion nuclear magnitude derived for the comet ($r > 8$ AU) to calculate an effective radius of the nucleus (R), which for plausible values of the geometric albedo, $p_v \sim 0.03$ –0.6, lies in the range $R = 1$ –4 km.

Pre-perihelion spectrophotometric observations (wavelength $(\lambda) = 3,000$ –7,000 Å, $\lambda/\Delta\lambda \sim 250$) covering a heliocentric distance (r) in the range 5.6–4.2 AU of comet P/Halley were obtained using the photon-counting Reticon spectrograph on the 4.5-m Multiple Mirror Telescope (MMT) of the F. L. Whipple Observatory (Table 1). A ratio of the spectrum of comet P/Halley with the solar spectrum is shown in Fig. 1, which provides direct evidence for a gas coma in the comet. The presence of the CN emission band (3,883 Å) is evident in the comet spectrum, confirming our previous report of detection of CN emission in the 17 February 1985 MMT data¹.

All the MMT spectra listed in Table 1 have been ratioed and differenced both with the solar spectrum² and with that of a solar analogue to search for weak cometary emission features. Comparison of the February and March spectra of comet P/Halley indicates that the emission feature at 3,883 Å is the only feature present above the 1.5σ detection level in both spectra at the same wavelength and with a width larger than one resolution element. No statistically significant detection of the CN band was found in the November 1984 spectrum. Only a marginal detection of CN emission was indicated in the April spectrum, which may be explained by the fact that the comet was observed through a considerable air mass and for a shorter integration time than in February and March. No other cometary emission features would be expected to be detectable.

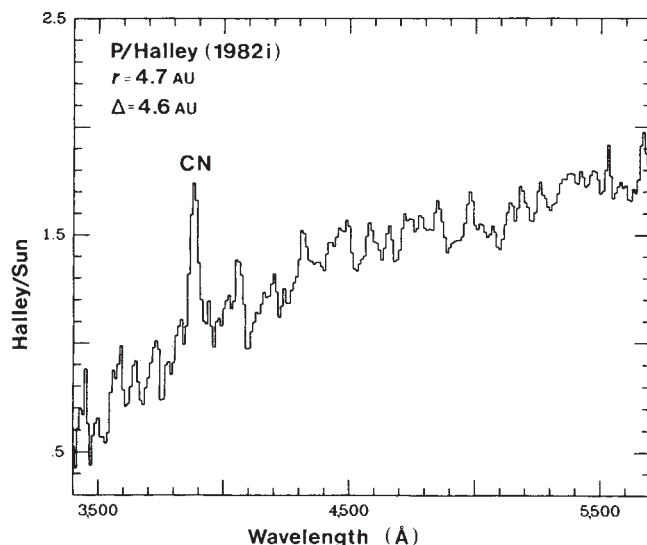


Fig. 1 Detection of the $\Delta v = 0$ band sequence of the violet CN system ($B^2\Sigma^+ - X^2\Sigma^+$) is illustrated in this averaged (February and March data) pre-perihelion MMT spectrum of P/Halley (1982i) which has been ratioed with the solar spectrum. The CN emission demonstrates the presence of a significant gas coma in the comet. This spectrum was binned in 10-Å intervals before division by the solar spectrum, which was also binned in 10-Å intervals. Note the colour difference, $\Delta(B - V) \sim +0.2$, in the sense (P/Halley–Sun) indicated by the slope of the ratioed continua.

Table 1 shows measurements and 2σ upper limits for the CN emission band fluxes obtained from the (Halley–Sun) difference spectra after normalization at the continuum near 3,883 Å. The CN band fluxes were converted to column densities, and CN production rates were calculated using the vectorial model³. In the calculation, photo-dissociation lifetimes ($\tau = 1$ AU) of 22,000 and 100,000 s were adopted for the CN parent and for CN, respectively. The assumed parent velocity was 0.8 km s^{-1} and that for CN 3.0 km s^{-1} . Assuming $Q(\text{OH})/Q(\text{CN}) \sim 500$, the production rate of H_2O in P/Halley at $r \sim 4.7$ AU is estimated to be $Q(\text{H}_2\text{O}) \sim 2 \times 10^{27}$ molecules s^{-1} .

Photometric data derived from the continua of the MMT spectra, together with published broad-band photometry of comet P/Halley, provide evidence for the onset of sublimation of the nucleus. To our knowledge this represents the first observation of the onset of sublimation in any comet. The V magnitudes, uncorrected for aperture effects and derived by convolving the MMT spectra with the standard V passband transmission function⁴, are listed in Table 1. The comet was visible on the MMT television monitor during the February, March and April observations. In March and April the average seeing diameter was ~ 1 –2 arc s and the images of P/Halley had soft appearances relative to stars in the same field, indicative of a resolved cometary image (angular diameter > 2 arc s). During these observations the comet was not seen on the television monitor more extended than 5 arc s to a surface brightness level of $V \sim 21$ –22 mag arc s^{-2} . The spectrum in November was obtained using a blind offset, as the comet was too faint to be seen on the television monitor.

Table 1 MMT Reticon observations of P/Halley (1982i)

Date (UT)	r (AU)	Δ (AU)	Φ °	Aperture* (km)	$V^\dagger$ (mag)	$V(1,0)$ (mag)	$F(\text{CN})$ (erg $\text{cm}^{-2} \text{s}^{-1}$)	$Q(\text{CN})$ (molecules s^{-1})
26.4 November 1984	5.60	4.76	5.6°	4,850	20.9	13.6	$< 1 \times 10^{-15}$	$< 6 \times 10^{25}$
17.2 February 1985	4.84	4.45	11.2°	8,050	19.9	12.8	$(3 \pm 2) \times 10^{-16}$	$(3 \pm 2) \times 10^{24}$
24.2 March 1985	4.50	4.73	12.1°	8,600	19.4	12.4	$(5 \pm 4) \times 10^{-16}$	$(4 \pm 3) \times 10^{24}$
18.2 April 1985	4.25	4.87	10.0°	8,850	18.7	11.8	$< 2 \times 10^{-15}$	$< 1 \times 10^{25}$

* Projected circle equivalent radius of 2×3 arc s aperture in November; projected radius of 5 arc s diameter circular aperture in February, March and April.

† Estimated internal error is ± 0.3 mag. Both magnitude and flux measurements for February data supercede those given in a preliminary report¹.

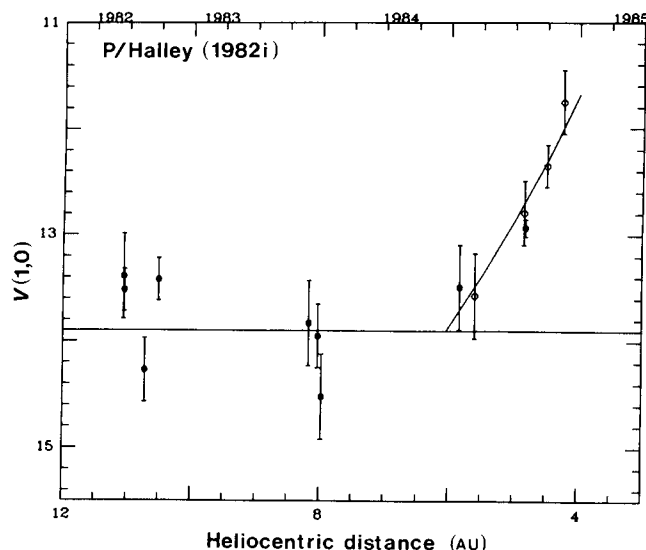


Fig. 2 Evidence for the onset of sublimation of the nucleus of comet P/Halley near $r \sim 6$ AU. $V(1,0)$ is the V magnitude of the comet reduced to a distance 1 AU from the Earth, 1 AU from the Sun and to a direction directly opposite the Sun. An inactive comet nucleus would have a constant $V(1,0)$ magnitude. Dates at the top represent mid-year epochs.

To remove brightness variations resulting from changes in the heliocentric and geocentric distances of the comet, the V magnitudes were converted to reduced magnitudes using

$$V(1,0) = V - 5 \log r - 5 \log \Delta - 0.039 \Phi \quad (1)$$

where $V(1,0)$ is the magnitude of the comet at $r = \Delta = 1$ AU, V is the observed magnitude (Table 1), Φ is the solar phase angle and an asteroid phase function, 0.039, is assumed. In Fig. 2 the reduced magnitudes of P/Halley are plotted against the heliocentric distance; photometry published elsewhere⁵⁻¹¹ has also been included. An inactive cometary nucleus would be expected to have a constant $V(1,0)$ (except for the effects of rotation and aspect).

There is clear evidence for an increase in the brightness of the comet at $r \sim 6$ AU, indicative of the development of a dust coma (see Fig. 2). Significant continuous sublimation of the nucleus of P/Halley commenced sometime between mid-1984 and early 1985 (Fig. 2). This onset epoch is consistent with the observation of an extended coma reported in late September 1984 by Djorgovski and Spinrad¹². In fact the September appearance of the extended coma associated with comet P/Halley could have resulted from a rupture of a surface dust mantle because of solar heating after the 75-yr incubation period of the comet. Note that the lack of CN in the November spectrum (Table 1) does not preclude the estimated sublimation onset epoch, as CN emission would have been undetectable (at the 1.5σ level) with the MMT Reticon spectrograph even if the November band strength had been comparable with that observed in February 1985.

The constancy of the mean value of the reduced magnitude for $r > 8$ AU indicates that systematic sublimation was not occurring in comet P/Halley from $r \sim 12$ to 8 AU. Hence, we conclude that the observations obtained when the comet was located at $r > 8$ AU represent the inert nucleus. For $r > 8$ AU we calculate the mean reduced nuclear magnitude of the comet, $\overline{V(1,0)}$, to be 13.9 ± 0.5 mag, where the error represents the r.m.s. deviation in the data. The intersection of the sublimation curve with the $\overline{V(1,0)} = 13.9$ line in Fig. 2 gives a heliocentric distance corresponding to the onset epoch, $r \sim 6 \pm 1$ AU.

The photometric observations of comet P/Halley are plotted as a function of heliocentric distance in Fig. 3. The secular variation of the nuclear magnitude of comet P/Halley with heliocentric distance can be explained adequately by changes in heliocentric and geocentric distances and phase angle [equation (1)] during the interval 1982–85 ($r = 12$ –6 AU). A

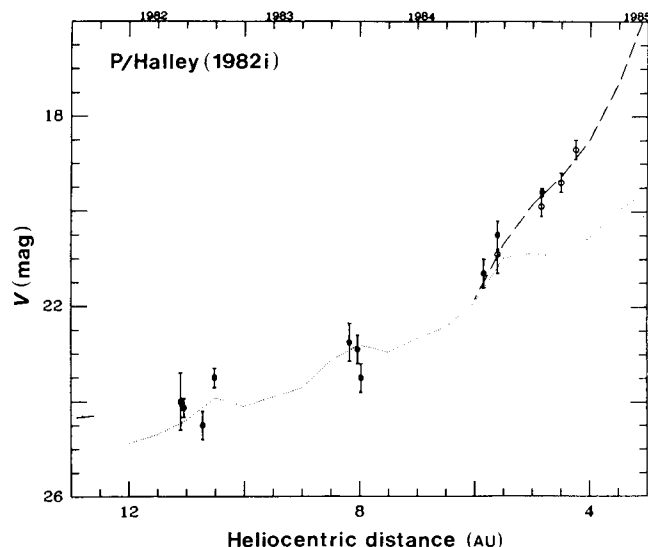


Fig. 3 Light curve of comet P/Halley (1982–85) showing the predicted nuclear magnitude (equation 2; dotted line) and the predicted total magnitude ($V \sim r^{-7 \pm 3}$; dashed line). The secular increase in brightness of comet P/Halley can be explained within the observational uncertainties by relative changes in distance (and phase angle) between the Earth, Sun and comet until the comet reached a heliocentric distance of $r \sim 6$ AU, when systematic brightening indicative of the dust coma development.

curve fitted to the photometric observations after the onset of sublimation indicates an initial dependence of the sublimation rate $V \sim r^{-7 \pm 3}$.

Figures 2 and 3 show that the photometric data obtained from comet P/Halley before $r \sim 8$ AU probably refer to the bare nucleus. Hence, an effective radius can be calculated using the mean reduced magnitude, from

$$p_v R^2 = k 10^{(0.4[V(\odot) - \overline{V(1,0)}])} \quad (2)$$

where $k = (1.5 \times 10^8 \text{ km})^2 / \pi$, p_v is the geometric albedo ($\lambda \sim 5,500 \text{ \AA}$), R is the effective (circle equivalent) radius of the nucleus, $V(\odot)$ is the apparent visual magnitude of the Sun (-26.7) and $\overline{V(1,0)}$ is the mean reduced magnitude of the comet nucleus, 13.9, derived above. We find for comet P/Halley

$$p_v R^2 = 0.5 \pm 0.2 \text{ km}^2 \quad (3)$$

which differ slightly from a previous determination by Hughes¹³.

As the albedo of the comet nucleus is not yet known, we adopt a plausible range of albedos, $p_v = 0.03$ –0.6, which spans low-albedo asteroids to the higher-albedo icy surfaces such as the jovian satellites. For this albedo range, we calculate an effective radius for the nucleus of comet P/Halley, $R = 1$ –4 km. Note that if there is a significant contribution to the $\overline{V(1,0)}$ magnitude from a particulate halo surrounding the nucleus, then this range represents an upper limit to the effective radius of the solid-body nucleus of comet P/Halley.

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Interplanetary conditions during 3-kHz radio-wave detections in the outer heliosphere

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A very important observation¹ regarding the plasma physics of the outer heliosphere has been that plasma-wave instruments on both the Voyager 1 and 2 spacecraft beyond ~12 AU detected, during the same interval of time, plasma waves (frequency ~3 kHz) that could possibly be associated with the turbulence expected at the heliopause. It was suggested that this turbulence had propagated inwards to the spacecraft, which would place the heliosphere boundary at ~46 AU. To interpret these unusual observations, it is important to understand the characteristics of the interplanetary medium at large heliocentric distances. We draw attention here to the low-energy charged-particle environment in the outer heliosphere during the observations of the unusual plasma-wave signals. These particle data suggest that the outer heliosphere was unusually stable and free of transient shock and particle events for the ~8 months during the wave observations.

Figure 1 shows the counting rates as a function of time for protons (~3–17 MeV) measured by the Low Energy Charged Particle (LECP) experiment² on both Voyager spacecraft from 1982 to 1984. The plotted data are 3-day averages. The dotted line on Fig. 1 is day 243, 1983, when the unusual plasma waves were first observed¹. The wave signals are reported to have disappeared in early spring 1984 (ref. 3). During this interval of 1983–84 (which we subsequently refer to as the signal period, SP) the two Voyager spacecraft were at approximately the same helio-longitude, with Voyager 1 increasing in helio-latitude from ~20.5° to ~22.5° and in distance from ~17.2 to ~19 AU while Voyager 2 remained approximately in the ecliptic plane, moving from ~12.5 to 13.7 AU (ref. 1) on its way towards encounter with Uranus in January 1986.

During the SP interval, Voyager 1 measured no energetic protons for about 200 days. Before day 243, 1983, and after about day 75, 1984, many transient proton enhancements were observed. Voyager 2, in the ecliptic plane, observed some proton activity at about day 270, but, in general, the activity in the SP interval was much suppressed compared with that before day

243, 1983, and after day 75, 1984. Examination of Voyager LECP ion data (~50 keV) shows that the only activity during the SP interval was from co-rotating interplanetary shocks⁴. This conclusion was also reached by the Voyager cosmic-ray instrument team studying >0.5-MeV protons⁵.

We suggest that this ~200-day interval of quiescence in the energetic ion population in the outer heliosphere was directly related to the fact that the anomalous plasma waves (waves at frequencies above the local plasma frequency in all cases) were observed. We suggest that the interplanetary medium at this time consisted only of outwardly propagating co-rotating shocks, providing a rather stable interplanetary environment for the propagation (and possibly generation) of plasma waves. No transient shocks from solar flares overtook the co-rotating shocks to disrupt the stability of the medium. Indeed, the medium may well have resembled, for ~200 days, the prediction of Burlaga⁶, who speculated that beyond ~10 AU the large-scale dynamics of the interplanetary medium could be dominated by pressure waves; the originating coronal signatures would be lost. The solar wind would, therefore, be more homogeneous on a large scale. We suggest that the dashed line in Fig. 1 marks the end of the wave observations in early spring³.

The basic question then arises as to the origins of the plasma waves detected during this interval by the two Voyager spacecraft. We note that in a stable medium consisting only of co-rotating shocks, the plasma waves, if generated at the plasma frequency at the heliosphere boundary, could propagate to the spacecraft in a medium undisturbed by disrupting transient events. The propagation could be across the shocks or along the spiral field configuration between the shocks. Further, the absence of energetic protons at both spacecraft for such a long interval implies the absence of such protons at the heliopause. Such energetic protons in association with transient shocks, in the turbulence of the heliopause, could disrupt the plasma-wave generation processes there. Alternatively, the observations of the unusual plasma waves during this time interval could arise from plasma-wave generation at about twice the local plasma frequency, at the co-rotating shocks, with the waves channelled preferentially between the shock structures in the unusually stable interplanetary conditions.

While considerable study and modelling have been reported on the large-scale magnetic character of the inner heliosphere^{7–10}, less work has been reported on the heliosphere structure beyond ~10 AU and, in particular, on the propagation of heliosphere plasma waves in the various magnetic structures formed by co-rotating shocks and/or by transient shock conditions. The possibility that the heliopause has been remotely detected during the stable interplanetary conditions reported here should stimulate work on studies of the propagation of plasma waves in the outer heliosphere in an ordered

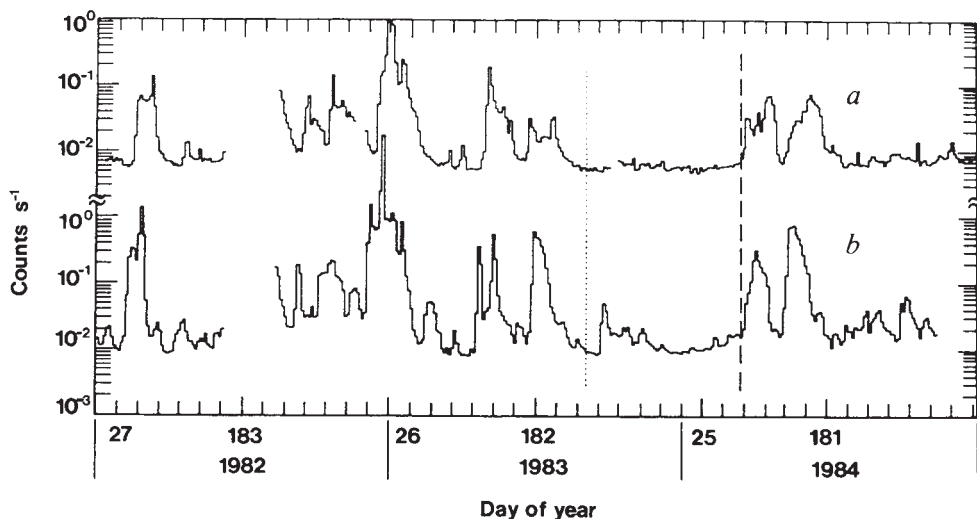


Fig. 1 Proton counting rates (3-day averages) measured on the Voyager 1 (a; 3.4–17.6 MeV) and Voyager 2 (b; 3.0–17.3 MeV) spacecraft from 1982 to 1984. The dotted line marks the onset of 3-kHz radio-wave observations by Voyager¹. The dashed line marks the expected termination of the detection of the waves determined from considerations of the energetic ion fluxes in the outer heliosphere.

interplanetary environment devoid of transient shocks and energetic ions.

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Characteristics of *in situ* coal gasification liquids from a cretaceous coal

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The concept of harnessing energy from fossil fuel by gasification of coal in the ground has been revived with the aim to increase usable coal reserves and to minimize the environmental impacts of mining and other problems. We report here the characteristics of liquids formed as a by-product of an underground gasification pilot test burn of a Cretaceous coal seam at a central Alberta location. The simplicity of the composition of liquids produced suggests that *in situ* coal gasification may be an economically viable source of refinery feeds or fuel.

During the summer and early autumn of 1976, an exploratory field test of underground coal gasification was carried out in the Battle River coal field, ~195 km south-east of Edmonton, on a 3.6-m zone of Cretaceous sub-bituminous coal. This coal zone is part of the Horseshoe Canyon Formation and lies almost horizontally under ~18 m of fairly impervious shale and clay. A rectangular 18.3×9.1 m four-well system was placed in the direction of the face cleat to facilitate the breaking of the coal according to its natural trend. The boreholes were so linked by surface piping that any one hole could serve for air injection or for delivery of gas through a knock-out tank (for stripping tarry materials from the production gas) to the flare stack. During the gasification segment of the test burn, ~300 tonnes of coal were affected and 25,500 m³ of gas with a heating value of 3,700 kJ m⁻³ produced. Excavation of the site after the test burn indicated that 50 tonnes of dry coal were pyrolysed and gasified. A substantial quantity of tar and light oil was collected at the knock-out tank while the gasification was in progress. The simplicity of composition of these liquids suggests that by-products of *in situ* coal gasification may be an economically viable source of refinery feeds or fuel.

To provide laboratory support for the field test programme, we designed a simulator which used a 1×1×2 m coal block retaining most of the essential features of an undisturbed coal seam, and several simulations of underground coal gasification (UCG) were performed. Detailed descriptions of the simulation experiments¹ and field tests²⁻⁴ have been published elsewhere.

An insight into the composition of the organic content of the liquid effluents from the field tests was gained by liquid-liquid extraction of an aliquot of the product sample with an organic solvent, followed by IR spectrometry on the extract. The spectra, showing the presence of strong —OH and —O— bands, together with well-resolved 1,600–1,800 cm⁻¹ bands, were almost identical to spectra obtained for low-temperature pyrolysis tar of

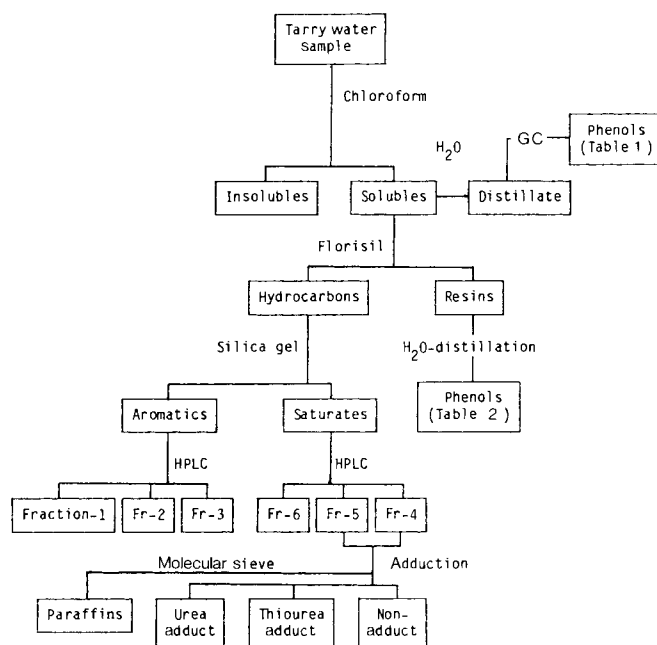


Fig. 1 UCG liquids separation scheme.

low-rank coals. As the IR spectra of field and laboratory test samples indicated the presence of carboxylic acid and acid anhydrides, more detailed analyses of the liquid effluents collected as spot samples from the slipstream of the product line, from the knock-out tank and from the slipstream before the flare stack, were carried out as the field test progressed.

The final separation scheme (Fig. 1) was as follows: the samples were exhaustively extracted with chloroform; the solids were removed by filtration, and after removing chloroform from the filtered chloroform layer, a fraction of the residuum was separated into 'hydrocarbon' and 'resin' fractions by elution chromatography on a Florisil column. The resin fraction was steam-distilled and the steam-volatile fraction analysed for phenols by gas chromatography. The hydrocarbon fraction was separated on silica gel to give 'saturates' and 'aromatics', and each of these was re-separated by HPLC on amino phase (Lichrosorb-NH₂) using *n*-hexane eluent. HPLC separation showed that the 'saturates' from silica gel were contaminated with a small amount of 'aromatics' and these were removed as HPLC fraction 6. The HPLC 'saturates' fractions 4 and 5 were combined and further adducted with urea and thiourea to give urea-adduct, thiourea-adduct and thiourea-nonadduct following standard procedures⁵⁻⁷. Part of the 'saturates' was adducted on a molecular sieve (5 Å).

A large proportion of the steam-volatile fraction was identified as phenols (Table 1). The first member (phenol) of the homologous series was by far the most abundant constituent, followed by cresols (meta-, *m*-; para-, *p*-; and ortho-, *o*- in that order), while the remaining higher phenols were present only in minor proportion. The distribution of phenolic compounds remained constant during the steady burn period. A build-up of trimethyl phenols was noted in the product stream. Between 20 and 25% of total phenols escaping into the flare stack were identified as trimethylphenols on the fourth day of the burn period (sample F-4-3 and F-4-3₂).

The relative concentrations of the classes of compounds separated on the Florisil and silica column are shown in Table 2. The percentages of the chloroform-soluble liquids from effluents in steady burn conditions were 30% saturates, 25% aromatics, and 19% phenols; complex phenols plus compounds of high relative molecular mass (noted as non-volatile resin, Table 2) made up the last 26%. The error of analyses was ±3–5%. The hydrocarbons representing >50% of the UCG liquids were separated into saturates and aromatics on silica gel, and into six fractions

Table 1 UCG field test: phenol contents and distribution in liquid product effluent

Sample	F-1-1	F-1-2	F-4-3	F-4-3*	F-4-3 ₂	F-4-3 ₂ *
Wt % organics						
Stem-volatile fraction	50.0	68.3	15.0		15.0	
Phenols	32.9	55.3	8.3		7.8	
% Composition of phenols						
Phenol	43.0	45.0	13.0	13.8	13.1	14.2
<i>o</i> -Cresol	7.6	6.8	5.5	5.5	5.2	2.9
<i>m</i> -Cresol	20.7	20.6	14.3	15.8	14.2	17.0
<i>o</i> -Ethylphenol	0.7	0.5	1.5	1.5	1.3	0.4
<i>p</i> -Cresol	9.2	9.5	9.3	11.0	8.4	11.0
<i>m</i> -Ethylphenol	2.2	2.1	3.1	3.4	3.3	4.4
<i>p</i> -Ethylphenol	2.6	2.4	5.5	5.2	4.9	6.7
Dimethylphenol 2,6-	0.6	0.1	1.9	1.3	2.0	0.9
2,4- and 2,5-	3.2	2.2	8.6	7.9	7.6	3.9
2,3- and 3,5-	5.2	4.6	8.1	7.2	8.0	8.7
3,4-	3.3	2.4	6.0	4.7	7.0	7.4
Trimethylphenol 2,4,6-	2.3	2.2	8.9	3.3	10.7	10.0
2,3,6-	?	1.6	11.2	17.8	13.0	10.1
2,3,5- and 2,4,5-	?	?	3.2	1.4	1.1	2.4

* Phenols separated by carbonate wash. F-1-1, sample from day-1/slipstream before knock-out (KO) tank; F-1-2, sample from day-1/KO tank; F-4-3₂, sample from 2nd test day-4/slipstream after KO tank; F-4-3₂², sample from day-4/slipstream after KO tank (1st test).

by HPLC. Preliminary characterization of the HPLC aromatic fractions came from a comparison with the retention times of a standard aromatic hydrocarbon mixture (benzene, naphthalene and phenanthrene); this suggested that fraction 1 consisted of mono- and di-aromatic hydrocarbons, fraction 6 mostly di-aromatic, and fraction 2 contained di- and tri-aromatic hydrocarbons only. More complex materials were present in fraction 3. Probable structures and compositions of all separated fractions were derived from subsequent analysis by gas chromatography and mass spectrometry (GC-MS).

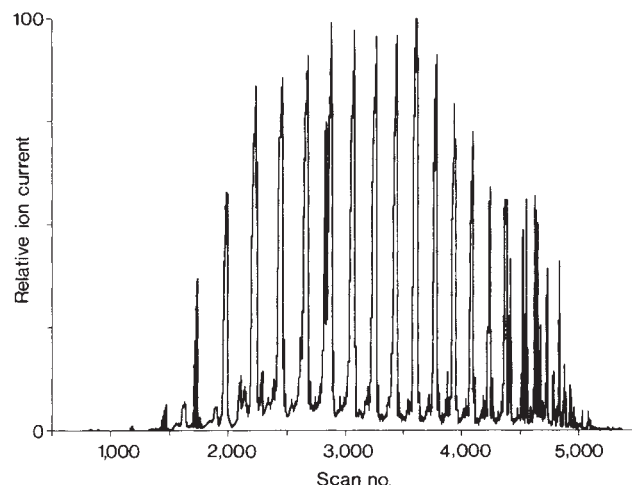
The first major peak in fraction 1 was identified as naphthalene by GC-MS analysis. Subsequent peaks were methyl, dimethyl and trimethylnaphthalenes. Also, in the same chromatogram, a series of minor peaks can be observed between scan 2,000 and 3,000 belonging to acenaphthenes and possibly biphenyls. Fraction 2 seemed to be an intermediate zone between di- and poly-aromatics. Here the major group of compounds were dibenzofurans with minor amounts of acenaphthenes and biphenyls. The series of molecular ions $M+168$, 182, and so on, represented dibenzofurans, while the isobaric acenaphthenes were concentrated in fraction 1. The higher members of the homologous series were the corresponding di- and trimethyl derivatives.

Fraction 3 was spectroscopically resolved into two groups. The first group was identified as anthracenes-phenanthrenes from the first member of the series to their tetramethyl homologues. No attempt was made to distinguish between anthracenes and phenanthrenes. The second group has the two largest peaks represented by pyrene and fluoranthene, together with smaller amounts of their monomethyl homologues. Careful extraction of mass spectral data for scan 3,500 and 4,000 indicated that a smaller amount of chrysene and a minute quantity of perylene were also present in this fraction. The total ion count

Table 2 UCG tar composition according to separation scheme (wt % distribution)

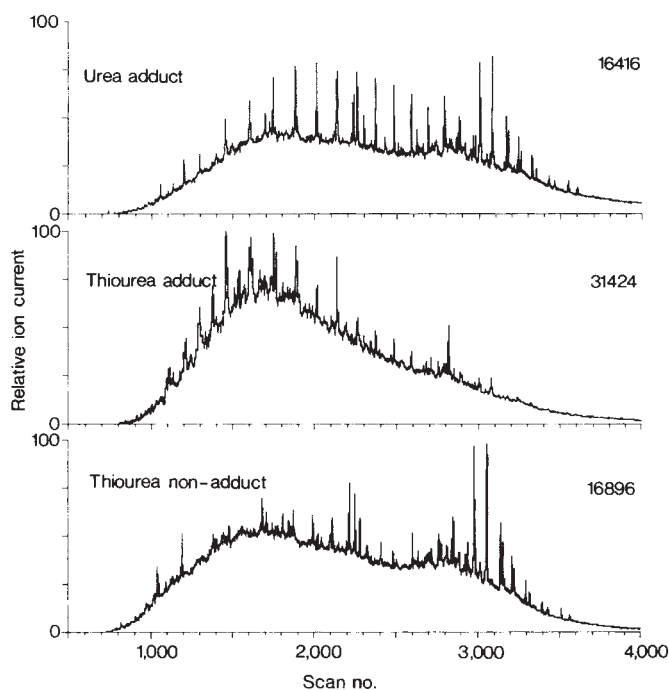
Sample	F-3-1*	F-3-2*	F-4-3	F-4-3 ₂ (tar only)	F-4-3 ₂ (tar in water)
Particulates					
+asphaltenic matter	12.5	6.6	3.7	5.3	4.3
Saturates	31.2	26.6	19.4	28.1	23.6
Aromatics	18.7	16.6	20.2	30.4	28.5
Steam-volatile resin	12.5	26.6	11.7	10.7	26.8
Non-volatile resin	18.1	20.0	36.9	22.5	17.3
Loss	7.0	3.6	8.1	3.0	—

* F-3-1, sample from day-3/slipstream before KO tank; F-3-2, sample from day-3/KO tank.

**Fig. 2** Mass chromatogram of saturate fractions.

(TIC) chromatograms provided an approximate distribution pattern, the relative order of abundance being anthracene-phenanthrene followed by pyrene-fluoranthene, di- and tetramethyl naphthalenes, methylnaphthalene, acenaphthene/biphenyl, dibenzofurans, chrysene and acenaphthenes.

Figure 2 is the TIC chromatogram of the combined saturate fractions 4 and 5. Here the peaks are equidistant; superimposed on them are two series of peaks—one at a lower scan and the other at a scan number from 4,100 to the end of the chromatogram. The identity of the group of major peaks was confirmed as a homologous series of *n*-paraffins. C_{14} – C_{24} paraffinic hydrocarbons were the main constituents. After the removal of paraffins on a molecular sieve, the sample was separated into urea and thiourea adducts and thiourea nonadduct (Fig. 3). The three fractions of adduction show, respectively, a wide distribution of very small amounts of open-chain to pentacyclic hydrocarbons. The presence of sterane and hopane hydrocarbons together with smaller amounts of tetra- and tricyclic hydrocarbons was identified from simple cross-scans of the thiourea nonadduct fraction combined with a consideration of the retention time. The retention times correspond to C_{21} – C_{29} steranes and C_{27} – C_{34} hopanes.

**Fig. 3** Mass chromatogram of urea and thiourea adducts and of thiourea nonadduct (number in upper right is total ion count).

Thus the composition and compound types present in the UCG liquid from a Cretaceous coal field were established. The paraffinic hydrocarbons, hopanes and steranes, along with aromatic hydrocarbons and phenols, represented >80% of all constituents. The abundance of the hydrocarbons was too high to discount them as coal seam contaminants; it is more likely that these are an integral part of the 'coal molecule' or are present in the coal as 'inclusion compounds'⁸. Deno and co-workers⁹ obtained long-chain paraffinic acids by selective oxidation of various types of coals by trifluoroacetic acid oxidation. The simplicity of composition of the UCG liquids suggests the economic potential of this liquid for use as refinery feed or fuel.

A major omission of the evaluation of field tests and simulation experiments was the absence of quantification of liquid yield per tonne of coal. After excavating the test sites, it was observed that the gasification zones in the test burns always followed a pyrolysis zone containing disintegrated pyrolysed char retaining no trace of the cleat orientation. The temperature of this zone was below 600 °C as revealed by an analysis of the char⁴. It is then reasonable to assume that the UCG liquids were formed predominantly by pyrolytic reactions, and contained the most stable and steam-volatile components. An optimum estimate of liquid yield could thus be expected from the Fischer-Schrader assay of this coal. The 50 tonnes of dry coal (affected in the test burns) gave 15 tonnes of ASTM (American Standard for Testing Materials) volatiles which contained 5 tonnes of Fischer gas, 3 tonnes of product water and 7 tonnes of Fischer light oil and tar. The leftover carbon in the pyrolytic char was gasified to give 16 tonnes of carbon monoxide (~13,000 m³).

Steam-volatility of UCG liquids may cause contamination of the aquifer and create new ecological concerns. The close monitoring of groundwater around the Alberta test zone over a 2-yr period did not show any phenolic contamination to a detectable level in the groundwater. A continuous large-scale operation has to be monitored more thoroughly.

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Synthetic seismogram modelling of the oceanic P_n phase

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The seismic oceanic P_n phase is characterized by low spatial attenuation, a high-frequency wavetrain of extremely long duration and an onset velocity near 8.0 km s⁻¹. In an effort to explain these properties, models have been proposed for the mode of propagation of this phase which include energy entrapment within a low-velocity channel^{1,2}, propagation within an extremely low-attenuation lithosphere waveguide^{3,4} and forward-scattering from local heterogeneity within the oceanic crust^{5,6}. Through synthetic seismogram modelling, we have generated realistic oceanic P_n phases for an oceanic lithosphere model that did not contain any of these intrinsic properties. The synthetic P_n phase can be explained rather simply as a set of refractions from the lower lithosphere with

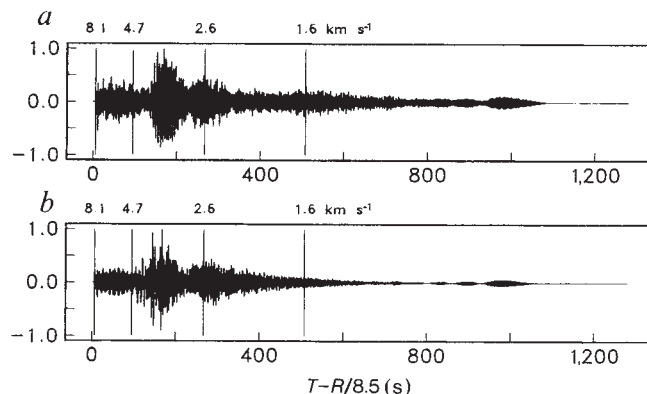


Fig. 1 Complete synthetic seismograms at an epicentral range, R , of 1,000 km for a thrust fault source at 14.0 km depth. The fault had a dip of 45°, a rake of 270° and an azimuth relative to the receiver of 60°. *a*, Vertical component; *b*, horizontal component rotated 25° clockwise from the radial direction. Group velocities of 8.1, 4.7, 2.6 and 1.6 km s⁻¹ are indicated. Phase velocities between 3.85 and 25.0 km s⁻¹ were included in the calculation.

subsequent reverberations within the oceanic water column and sediment layer. Many of the spectral properties associated with layer reverberation observed in the synthetic P_n phase are revealed in data collected in the south-west Pacific. Complicated oceanic lithosphere models are not required to explain the gross characteristics of P_n propagation.

The realistic oceanic P_n phases were synthesized using wavenumber integration⁷. The technique involves a quadrature over wavenumber at fixed frequency points with the result then Fourier transformed into the time domain. The resulting seismograms contain all reverberations, including those off the free surface, as well as all P to S (primary to secondary) and S to P mode conversions. Although the algorithm is similar to the widely used reflectivity method⁸, it differs in several important respects. The inability of reflectivity to incorporate reverberations and mode conversions outside a 'reflection zone' that cannot contain the free surface renders the method inadequate for the study of P_n propagation. We will show that near-receiver sediment and water reverberations are quite important in the synthesis of oceanic P_n wavetrains and, therefore, that wavenumber integration is well suited for the study of this phase.

The most serious limitation of wavenumber integration is the restriction to models consisting of a stack of laterally homogeneous layers. We have chosen a model representative of normal oceanic lithosphere, consisting of a vertically inhomogeneous oceanic crust and upper mantle overlain by a 4-km-thick water layer and a 500-m-thick sediment layer⁹. The values of the quality factor, Q , chosen in agreement with seismic refraction studies¹⁰, were <500 for compressional waves in the crust and upper mantle and 50,000 in the oceanic water column. A low-velocity channel was not included and the seismic velocity was a monotonically increasing function of depth.

Figure 1 shows complete synthetic seismograms generated for a sub-Moho thrust fault at an epicentral range of 1,000 km. The vertical component is illustrated in Fig. 1*a* and the horizontal component in Fig. 1*b*. The division of these seismograms into two groups facilitates the schematic identification with the oceanic P_n and S_n phases. The group with an onset velocity near 4.7 km s⁻¹ (Fig. 1*b*) is grossly identified as the oceanic S_n phase. The first-arriving energy within this group is entirely comprised of horizontally polarized shear waves and is absent from the vertical component. The absence of a clear SV body wave at a velocity near the mantle shear velocity, 4.7 km s⁻¹, may indicate that the mantle shear Q used to calculate the synthetics, 225, was too low. Work is continuing in an effort to synthesize more realistic S_n phases.

The first-arriving group of energy in Fig. 1 has an onset velocity of 8.1 km s⁻¹, a duration of the order of 100 s and extends into

the onset of S_n . This group is characteristic of the oceanic P_n phase.

The importance of reverberations within the oceanic water column and sediment layer is most effectively demonstrated in the frequency domain. It is simple to show that the power spectrum of a wavelet reverberating in a single layer contains spectral peaks at frequencies given by

$$f_n = (2n+1) \frac{df}{2}, \quad (n=0, 1, 2, \dots) \quad (1)$$

where $df = 1/T$ and T is the time between multiples. In the case of near-vertical incidence, T can be approximated by the two-way travel time in the layer and equation (1) represents the eigenfrequencies for an organ-pipe mode¹¹. The restriction of P_n to rays with turning points in the sub-Moho portion of the oceanic lithosphere requires phase velocities to be bracketed by the sub-Moho compressional velocity and that at the base of the lithosphere. In the case of the synthetics, this range is between 8.1654 and 8.2038 km s⁻¹. The resultant rays are quite steep at the receiver, justifying the use of the two-way travel time in equation (1). The $df/2$ offset of the spectral peaks is due to a π -phase shift between multiples¹². The width of the spectral peaks at frequencies given by equation (1) is governed predominantly by the impedance contrast at the boundaries of the layer. Thus the peaks associated with water reverberation, which actually occur between the free surface and the base of the sediments, are quite narrow. The sediment layer, on the other hand, acts as an extremely leaky waveguide for compressional waves because of the low impedance contrast at the ocean-sediment interface. This, coupled with the low value of Q in the sediments, results in broad spectral peaks for sediment reverberations. The power spectrum of a wavelet reverberating

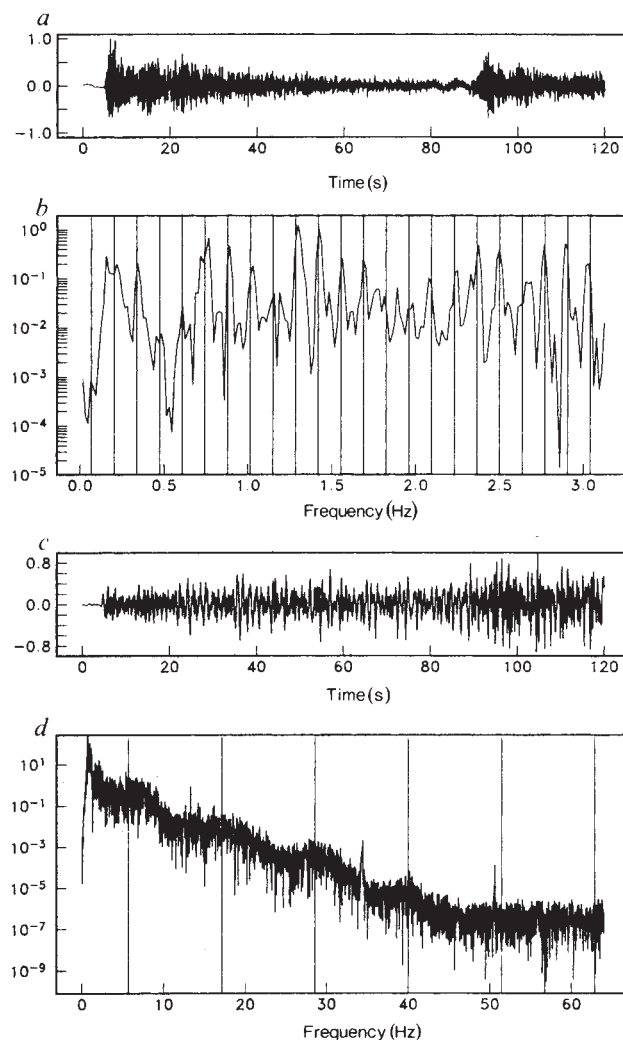


Fig. 3 Data collected in the south-west Pacific during the Ngendei Seismic Experiment. *a*, Hydrophone recording; *b*, the first 3.0 Hz of its corresponding power spectrum with vertical lines at predicted frequencies of water reverberation peaks. *c*, OBS vertical-component seismometer data and *d*, its corresponding power spectrum. Vertical lines indicate predicted positions of spectral humps associated with P-wave sediment reverberation.

in the oceanic water column and sediment layer thus consists of widely spaced spectral 'humps' due to sediment reverberation superimposed on finely spaced spectral 'peaks' associated with water reverberation.

Figure 2 illustrates this behaviour in the synthetic P_n power spectrum. Figure 2*a* shows the synthetic P_n wavetrain of Fig. 1*a*. The horizontal component power spectrum in Fig. 2*c* shows vertical lines indicating predicted positions of spectral humps associated with S-wave sediment reverberation. Figure 2*b* is the first 3.0 Hz of the vertical-component spectrum with lines at the predicted frequencies of water reverberation peaks. Figure 2 thus clearly illustrates the presence of these reverberations in the synthetic P_n wavetrain.

Data were collected during the 1983 Ngendei Seismic Experiment by an ocean bottom seismometer (OBS) array in the south-west Pacific (23°49' S, 165°32' W), ~900 km east of the Tonga trench¹³. Each OBS contained one vertical and two horizontal seismometers and a hydrophone. Figure 3 shows an example of these data along with their corresponding power spectra.

A clear feature of the P_n phase in Fig. 3*c*, also seen in the synthetic P_n phase of Fig. 2*a*, is a predominance of low frequencies late in the wavetrain. Modelling the decay rate of the energy in the signal pictured in Fig. 3*c* as an exponential of the form $e^{-\gamma t}$ for three separate frequency bands, each two octaves wide,

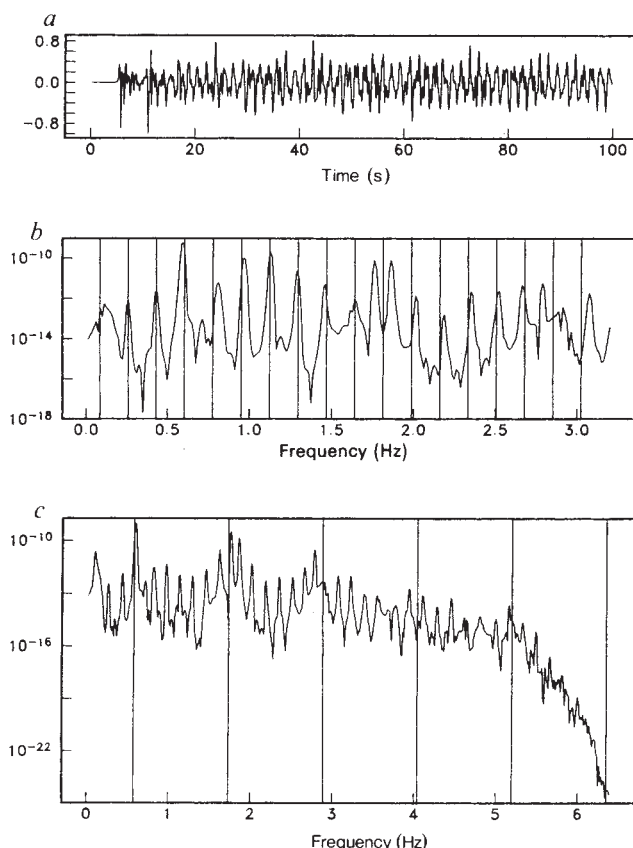


Fig. 2 Power spectrum of the synthetic P_n wavetrain. *a*, Synthetic P_n wavetrain in Fig. 1*a*. *b*, First 3.0 Hz of the vertical-component power spectrum with vertical lines at frequencies associated with water reverberation. *c*, Horizontal component spectrum with vertical lines indicating predicted positions of spectral humps associated with S-wave sediment reverberation. The synthetics were limited to a Nyquist frequency of 6.4 Hz due to the high computational costs involved.

resulted in values of $\gamma = -0.005$ between 0.5 and 2.0 Hz, $\gamma = 0.003$ between 2.0 and 8.0 Hz and $\gamma = 0.055$ between 8.0 and 32.0 Hz. This result is in direct contrast to models of forward-scattered coda⁶ which predict the fall-off rate to be a decreasing function of frequency. This feature can, however, be explained as the superposition of different modes of reverberation.

The hydrophone is a more narrow band instrument than the OBS vertical component seismometer and is better able to isolate the water reverberations and their corresponding spectral peaks as depicted in Fig. 3b. The vertical-component seismometer, on the other hand, can record the dominant frequency associated with sediment reverberation and clearly exhibits the corresponding spectral humps in Fig. 3d. The predicted frequencies of spectral peaks in both Fig. 3b, d were calculated using values of the sediment and water layer velocities and thicknesses obtained in an independent study¹⁴. When comparing the synthetic spectrum (Fig. 2c) with the data spectrum (Fig. 3d), it is important to realize that the synthetics were computed up to a Nyquist frequency of only 6.4 Hz whereas the data extend to 64 Hz. Thus the water reverberation peaks, which are separated by <0.2 Hz, when plotted on a scale extending to 64 Hz, appear rather incoherent as in Fig. 3d. To illustrate sediment reverberation in the synthetic spectra, we used a thick sediment layer to reduce the peak spacing. In the synthetics, the spacing between sediment reverberation humps is 1.1548 Hz, while in the data it is 11.43 Hz.

The amplitude of the synthetic P_n phase was within a factor of 2 or 3 of the data amplitudes and decayed as $\text{range}^{-1.3}$, slightly faster than pure geometrical spreading. An event located by the National Earthquake Information Service (NEIS) with a published magnitude of 5.8 and moment of 2.6×10^{24} dyn cm was recorded by three of the OBSs at the Ngendei site. The event was a thrust fault source with an azimuth relative to the receiver of 90° at an approximate epicentral range of 920 km. The absolute amplitudes in the first 20 s recorded for this event ranged from 0.76 to 1.44 V. To obtain the maximum amplitude of the synthetic P_n phase, we used a thrust fault source with an azimuth relative to the receiver of 90° at an epicentral range of 900 km. The moment determined by NEIS was used and, after convolving with the instrument response, the resulting maximum amplitude was 0.420 V. Differences of this order can be easily explained in terms of differences in model parameters, fault orientation and focal depth.

The steep incidence of the rays within the oceanic water column and sediment layer implies that only very local changes in bottom topography can affect the propagation of P_n between a particular source and receiver. As long as the OBS is situated in locally smooth topography, the spectral characteristics of P_n mentioned above will be apparent. The main effect of an irregular bottom topography would be to reduce coherence between OBSs at different sites. If the sites are sufficiently separated as to allow changes in either water or sediment thickness and/or velocity, the resulting P_n coda would suffer a severe reduction in spatial coherence.

The oceanic P_n phase can be explained simply as a set of refractions from the lower lithosphere with subsequent water and sediment reverberations. Synthetic P_n wavetrains with durations of the order of 100 s were generated in the absence of scattering. Thus the propagation of the oceanic P_n phase does not require features of the oceanic lithosphere such as low-velocity channels, anomalously high Q values or thin high-velocity lenses, but rather can be explained in terms of simple models with monotonically increasing velocity-depth profiles containing no lateral heterogeneity.

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Stopping of earthquake ruptures at dilational fault jogs

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Palaeoseismic studies over the past several years have indicated that segments of certain major faults tend to rupture at fairly regular intervals in characteristic earthquakes of about the same size¹. This implies the presence of local structural controls which govern the nucleation and stopping of ruptures. Understanding rupture arrest is important, not only because it governs the size of characteristic earthquakes, but also because deceleration of ruptures results in the radiation of high-frequency energy leading to strong ground motion². I show here that rapid opening of linking extensional fracture systems to allow passage of earthquake ruptures through dilational fault jogs in fluid-saturated crusts is opposed by transient suctional forces induced near the rupture tips³. Rupture arrest may then be followed by delayed slip transfer as fluid pressures re-equilibrate by diffusion.

Mapping of rupture traces within major strike-slip systems has shown that they are generally discontinuous on a range of scales^{4,5}. In the San Andreas system, echelon segmentation of the fault trace is common, with the trace stepping abruptly from one side to another of fault zones up to ~ 2 km wide. High-resolution microearthquake studies have demonstrated that large irregularities observed in the surface fault trace often extend throughout the seismogenic zone to depths of 10 km or more⁶⁻⁸. The mechanical implications of echelon fault segmentation have recently been examined in a two-dimensional static stress analysis⁹. Two important cases are considered (Fig. 1); for ease of reference, irrespective of shear sense or faulting mode, they are designated dilational and anti-dilational jogs, depending on the tendency for areal increase or reduction respectively through the step region. For an anti-dilational jog (for example, left step under right-lateral shear), the elastic interaction between echelon fault segments increases both the mean compressive stress between the segments and the frictional resistance at the segment tips, inhibiting slip transfer across the

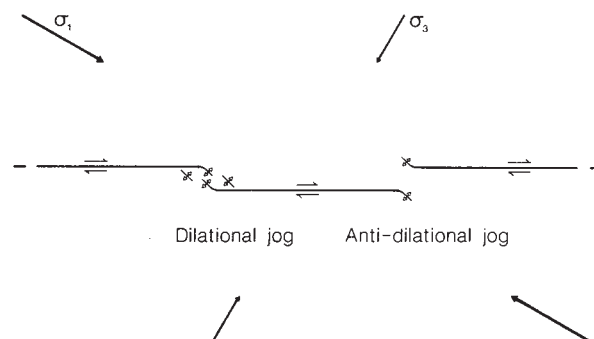


Fig. 1 Likely development of extension fractures in association with dilational and anti-dilational jogs along a segmented fault, shown in relation to far-field principal compressive stresses, $\sigma_1 > \sigma_2 > \sigma_3$ (after ref. 9).

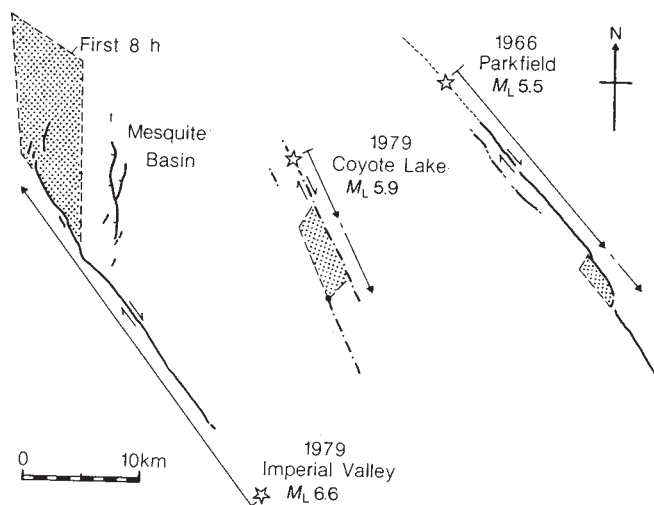


Fig. 2 Seismotectonic sketches illustrating the geometry of strike-slip earthquake ruptures within the San Andreas Fault system (stars, epicentre; arrows, propagation direction and extent of main-shock rupture; broad lines, surface breaks; dash-dot lines, micro-earthquake lineaments; stippling, rhomboidal areas of intense aftershock activity).

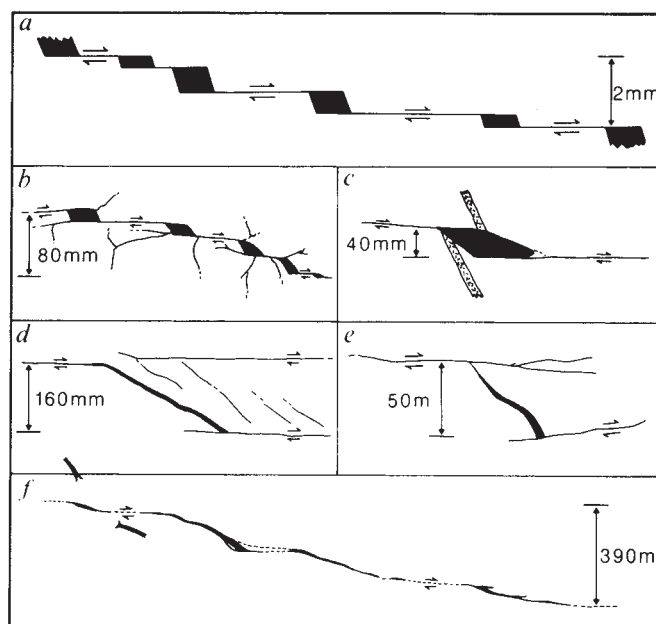


Fig. 3 Dilational jogs in various host rocks on a range of scales from natural fault systems (all drawn for dextral-shear sense). Black areas represent infillings of hydrothermal minerals or re-cemented breccias (a, quartz-chlorite fibrous infilling in metagreywacke²⁴; b, calcite infilling in flysch²⁵; c, quartz-epidote infilling in granodiorite²⁰; d, quartz infilling in granodiorite⁹; e, gold-quartz vein in greenstones²³; f, multiply quartz re-cemented breccia-vein in andesitic volcanics²²).

discontinuity. Such 'pinned' discontinuities form potential nucleation sites for moderate to large earthquakes^{7,9}. In contrast, for a dilational jog (for example, right step under right-lateral shear), frictional resistance at the segment tips is diminished, facilitating sliding, while mean compressive stress decreases throughout the inter-segment region, favouring the development of a linking fracture system involving extension and/or shear.

Thus, on the basis of this static analysis, echelon fault segmentation involving dilational jogs should present no major hindrance to the transfer of slip along fault systems⁹. Paradoxically, however, several recent strike-slip earthquake ruptures have terminated in the vicinity of dilational jogs which appear to have acted as kinetic barriers opposing rupture propagation. Seismotectonic sketches illustrating the rupture geometry in each case are given in Fig. 2.

The 1966 Parkfield earthquake (of M_L 5.5) is of particular interest as the latest in a series of characteristic $M_L \sim 5.5$ events that have previously ruptured the same Parkfield-Cholame segment of the San Andreas fault in 1934, 1922, 1901, 1881 and also possibly in 1857 as an immediate foreshock to the $M \sim 8$ event which ruptured through the "Big Bend"^{10,11}. Waveform similarities suggest that the last three events, at least, share essentially the same rupture characteristics, with the implication of strong controls on the nucleation and stopping of these characteristic events. Final ground breakage associated with the 1966 event extended for ~ 35 km (ref. 12). Structurally significant features along the trace include a slight restraining bend (equivalent to an anti-dilational jog) associated with the epicentre, and a pronounced ~ 1 -km dilational jog between echelon segments across the Cholame Valley. While dextral strike-slip at the surface eventually reached ~ 0.2 m near the centre of the rupture, most, if not all of it developed by afterslip over a period of 2 months following the main shock¹³. On the basis of seismic strong motion data and the aftershock distribution, it has been argued that the main co-seismic rupture was vertical, extended from 3 to 8 km depth and propagated unilaterally southeastwards for ~ 25 km from the epicentral bend, with a mean slip of 0.6 m, to terminate in the vicinity of the right-step¹⁴. The surface fault break was discontinuous across the jog, but aftershock activity was particularly intense beneath a 3.5×1 km rhomboidal area immediately north-west of its inferred position, indicating that the echelon segmentation persisted throughout the seismogenic regime to a depth of around 12 km (ref. 6). There are indications of relative subsidence within this rhombic area over a period of weeks following the earthquake¹². The 8-km fault break extending south-east of the jog was probably

entirely the product of post-mainshock afterslip which decreased logarithmically with time along with the aftershock activity¹³.

Information on the 1979 Coyote Lake earthquake (M_L 5.9) on the Calaveras Fault is based largely on high-precision aftershock studies and waveform analyses, because no clear surface break was observed. However, the seismological data indicate behaviour similar to the Parkfield event, with the main rupture propagating unilaterally southeastwards to terminate adjacent to a dilational jog delineated by intense aftershock activity. Mainshock slip averaging ~ 0.2 m was largely confined to a subvertical rupture extending from 3 to 10 km depth, which propagated southeastwards from the focus for 6–14 km (refs 15, 16). Some 5 h after the main earthquake, beginning with an M_L 4.0 event, aftershock activity extended ~ 2 km south-west to an echelon fault segment and migrated southeastwards over a period of days to define a subsidiary slip plane 8 km in length at depths between 3 and 7 km (ref. 8). Diffuse aftershock activity occurred within the overlap zone.

In the 1979 Imperial Valley earthquake (M_L 6.6), the rupture propagated unilaterally northwestwards for ~ 35 km with an average co-seismic slip of ~ 0.4 m, terminating at a larger-scale dilational jog defined by the down-thrown Mesquite Basin. Surface faulting occurred only on the northern three-quarters of the rupture along the Imperial fault, and also along the subsidiary Brawley fault. Detailed analysis of the main rupture on the Imperial fault¹⁷ shows that the amount of strike-slip, the slip rate and the rupture velocity diminish abruptly north of the intersection with the Brawley fault, but that the rupture continued on past a small restraining bend to stop finally where the strike of the Imperial fault veers northwards. In the first 8 h following the main shock, aftershocks were almost entirely concentrated beneath a 7×11 km rhomboidal area extending north from the main rupture termination¹⁸.

From their geomorphic expression, it seems clear that many of these dilational jogs are long-lived structures with substantial strike-slip motion transferred across them through some form of linking fracture system. Evidence on the nature of these fracture systems comes from several sources. Experimental work on brittle failure has demonstrated the strong tendency for cracks

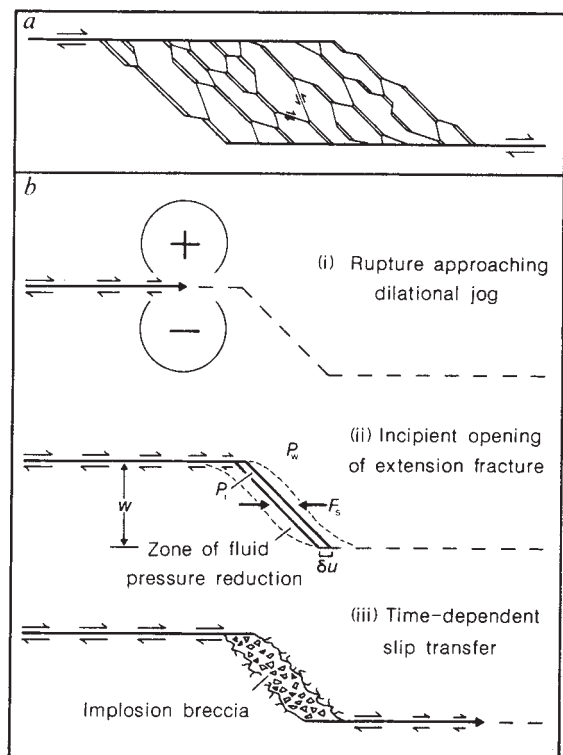


Fig. 4 *a*, Mixed extensional-shear fracture mesh model linking echelon fault segments across a dilational jog. *b*, Schematic representation of a dextral rupture with fields of compression (+) and dilatation (-) at the rupture tip encountering a dilational jog consisting of a single linking extension fracture (not to scale).

under shear (fault segments) to propagate into and terminate in, or become linked by extension cracks aligned perpendicular to the least principal compressive stress¹⁹; the same seems to hold true within natural fault systems²⁰. The diffuse, high b -value swarm seismicity sometimes associated with large dilational jogs has been satisfactorily modelled as resulting from a fracture system incorporating short shears linked by vertical extension fractures into a honeycomb mesh²¹ (see Fig. 4*a*). This is consistent with focal mechanisms obtained from aftershocks within the jogs which show a preponderance of strike-slip solutions with, rarely, some minor normal-slip^{6,8,21}. Smaller examples of dilational jogs, which have been described extensively on a range of scales from ancient fault zones^{9,20,22-25}, are also instructive. Echelon, planar fault segments tend to be linked through the jog regions by individual or multiple extension fractures, occasionally with subsidiary shears, or by the development of more extensive rhombic or lensoidal openings (Fig. 3). The linking fractures are commonly infilled by hydrothermal mineralization, forming extension veins, or contain breccias of wall-rock fragments cemented by hydrothermal minerals^{22,26,27}. Textural evidence often suggests a history of incremental opening for the extension veins, or of multi-episode brecciation and cementation^{22,26,28}. While they are clearly fault-related, the low clast/matrix ratios of these re-cemented breccias indicate significant dilation with included clasts of wall-rock remaining angular and showing little evidence for a derivation by frictional attrition²⁹. The important geometrical conclusion, however, is that the total extensional opening across the linking fracture system in the direction of fault movement is generally comparable to the slip on the echelon fault segments.

Assuming that the internal structure of the larger dilational jogs is analogous to that observed in these smaller-scale examples, we consider the problems associated with transferring co-seismic slip for moderate or larger earthquakes (>0.1 m, say) across such features if, as seems likely on comparative grounds, such slip transfer requires extensional opening of the same order within the jogs. Although the linking fracture system may resemble the mixed extensional-shear mesh model for

earthquake swarms²¹ (Fig. 4*a*), we represent it simply by an equivalent single extension fracture (Fig. 4*b*). While the deviatoric stress field within dilational jogs favours the opening of linking extension fractures⁹, static stress analysis does not consider problems associated with the rapid opening of such fractures in fluid-saturated crust. Throughout the seismogenic regime, fluid pressures may reasonably be assumed to be at least hydrostatic²⁸, increasing with depth at ≥ 100 bars km^{-1} . Unless the extensional opening takes place sufficiently slowly for the internal fluid pressures within the linking fractures (P_i) to remain the same as those in the wall-rock (P_w), the opening will be opposed by an induced suctional force (F_s) in the shear direction,

$$F_s = \Delta P w v \quad (1)$$

where ΔP is the average fluid pressure differential, ($P_w - P_i$), v is the vertical extent of the rupture and w is the width of the dilational jog (Fig. 4). Clearly, the magnitude of the induced pressure differential will depend on the porosity and fluid diffusivity of the surrounding rock mass. Fluids could enter the dilating fracture system either by channel flow along the fault segments or from wall-rocks adjacent to the fractures. The former can be neglected given the width of the jogs and the 1-10-s slip durations characteristic of moderate to large ruptures³⁰. At any depth within the seismogenic regime (approximately the top 10 km of crust for geothermal gradients of 25°C km^{-1}), the incompressibility of water is sufficiently high for a fluid volume increase $\leq 10\%$ to lower the fluid pressure to a negligible fraction of initial hydrostatic values³¹. For an infinite planar extension fracture opening an amount, δu , in a time, t , we can define an effective diffusive volume extending a distance, $L = (ht)^{1/2}$, each side of the fracture, where h is the fluid diffusivity of the wall-rock. If the wall-rock has a porosity, p , it follows that the conditions for creation of large fluid pressure differentials is

$$\delta u > 0.2 p L = 0.2 p (ht)^{1/2} \quad (2)$$

Taking a reasonable porosity of 5%, the 1-10-s range of earthquake slip durations, and estimates of fluid diffusivity in fault zones which range from $\sim 10^{-8} \text{ m}^2 \text{ s}^{-1}$ for impermeable clay gouge³² to bulk values³ of $1 \text{ m}^2 \text{ s}^{-1}$, δu must have minimum and maximum values of $1 \mu\text{m}$ and 30 mm , respectively, for large fluid pressure differences to develop. Because we are concerned with transferring slip >0.1 m across the jogs, an induced pressure differential approaching the initial hydrostatic value at any depth therefore seems likely, even if many extension fractures are involved in the linking system. With the tensile strength of rocks rarely exceeding 100 bar, it is easy to see how the high-dilation breccias described above may form by hydraulic implosion of the wall-rocks into extending fractures as a result of these transitory fluid pressure differences (Fig. 4) (see refs 27, 29). For a dilational jog extending to a depth of 10 km, the average pressure differential $\Delta P \approx 500$ bar and from equation (1), F_s scales with the width of the jog for ruptures occupying the entire seismogenic zone. The work done in opening linking extensional fractures against the resistive suctional force is

$$W_s = \delta u F_s = \delta u w v \Delta P \quad (3)$$

For appropriate values, $v = 10 \text{ km}$, $w = 1 \text{ km}$ and $\delta u = 0.1 \text{ m}$, $W_s = 5 \times 10^{13} \text{ J}$. From the Gutenberg-Richter relationship³³, this is comparable to the wave energy released by an earthquake of surface wave magnitude $M_s 6$ (similar to those under discussion), giving some idea of the extent to which the opening of extension fractures within a dilational jog may drain energy from a propagating rupture tip.

The difficulties in transferring earthquake slip across the larger jogs are thus apparent. Rapid opening of extension fractures is strongly opposed by induced fluid pressure differentials, while such drops in fluid pressure as occur within the jog cause dilatancy hardening, increasing effective normal stress and shear resistance on subsidiary shears. The stopping mechanism also provides an explanation for the observed time dependence of

slip transfer and aftershock migration across dilational jogs. Strain-energy concentrations around the end of the arrested rupture may be slowly dissipated by slip transfer across the jog as fluid pressures re-equilibrate by diffusion to allow the necessary extensional opening within the linking fracture system. Similar control of aftershock activity by fluid diffusion has been suggested before³⁴, but not at such specific structural sites. The range of possible fluid diffusivities, noted previously, is sufficiently large for the rate of slip transfer to vary considerably, as is observed. Time-dependent displacement and fluid transfer around the dilational jog at Parkfield should clearly be closely monitored following the next characteristic M_L 5.5 event, which is expected in 1988 ± 4 yr (ref. 10).

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Strontium content of ostracods indicates lacustrine palaeosalinity

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Individual non-marine ostracod valves contain Sr in proportion to the Sr content of their host lake water. Two commonly coexisting, large planktonic species, *Mytilocypris praenuncia* and *Australocypris robusta*, have a Sr distribution coefficient K_D (Sr/Ca) of 0.082 which is independent of temperature in the range 10–18 °C. If the relationship between salinity and the Sr content of a hydrologically simple lake is known, palaeosalinities can be calculated from fossil ostracods. We present here palaeosalinities for the past 10,000 yr for Lake Keilambete, Victoria, calculated from ostracod Sr/Ca of fossil material which are in striking agreement with independent interpretations of palaeosalinity. The range of salinity spans 14–95‰. We also show that a reliable quantitative continuous indicator of lacustrine palaeosalinity is important for the elucidation of continental palaeoenvironments for palaeoclimatology and archaeology and ocean–continent climatic correlations and interactions.

The rapid advance in the knowledge of Quaternary global climate, stemming largely from the study of Foraminifera from

the ocean basins^{1–3}, has not been matched by equivalent progress in continental environments. Lacustrine sediments can supply information on changing continental climate. Those techniques commonly applied to lacustrine sediments, including sedimentary facies analysis, palynology, documentation of biota (such as, ostracods and diatoms) with diagnostic salinity or temperature tolerances produce useful limiting ranges of palaeoenvironmental conditions. Oxygen-isotope studies of authigenic or biogenic lacustrine precipitates are commonly hampered by unknown previous $\delta^{18}\text{O}$ water compositions, which may have varied as a function of salinity (precipitation/evaporation ratio), precipitation regime and temperature and varying contributions from multiple fluvial and/or groundwater sources. What is lacking is a method capable of eliciting continuous salinity or temperature variations from lacustrine sediment cores.

Ostracods are considered to be ideal monitors of water quality because, before reaching maturity, they rapidly shed their shells several times (up to nine times, at approximately weekly intervals) during growth and the source material for their shells comes entirely from the host water⁴. We have shown previously⁵ that the Mg content of some non-marine ostracod shells is a function of salinity and temperature and that coupled Mg/Ca and $\delta^{18}\text{O}$ measurements might provide a record of combined palaeotemperature and palaeosalinity. However, the Sr/Ca ratios of the calcitic carapaces of the ostracod *Mytilocypris henricae*, grown in controlled conditions in aquaria and from natural lakes⁶, appear essentially independent of temperature (T) in the range 10.5–25 °C. Specimens of *A. robusta*, which is a species phylogenetically related to and of similar shape and size to *M. henricae*, collected at different seasons ($T = 10.2$ –18 °C) from Lake Keilambete in western Victoria (lat. 38°12'S; long. 142°53'E), share the same chemical relationship (Table 1). This raises the possibility that the Sr content of some non-marine ostracods may be used to determine palaeosalinity through the former Sr contents of the water.

A reconnaissance study of Sr/Ca ratios of carapaces from live ostracods from a series of south-east Australian lake waters⁵ of salinity 1.3–73‰ suggested that the Sr content of the shell was a direct and positive function of the Sr content of the lake water. We examined the Sr/Ca ratios of shells of 18 species, namely, *Australocypris* (two species), *Mytilocypris* (five species), *Diacypis* (three species), *Reticypris*, *Cyprinotus*, *Limnocythere* (three species), *Ilyocypris*, *Cypricercus* and *Cypretta*. We conclude that the distribution coefficient, K_D (Sr/Ca) = $[\text{Sr/Ca}]_{\text{CaCO}_3}/[\text{Sr/Ca}]_{\text{H}_2\text{O}}$, may be species dependent, although to a smaller degree than is the K_D (Mg/Ca) (ref. 5). The larger genera *Mytilocypris* and *Australocypris* have identical K_D (Sr/Ca). For modern *A. robusta* from Lake Keilambete (Table 1), K_D (Sr/Ca) ~ 0.082 .

For the first application of the Sr/Ca palaeosalinometer, we examined fossil ostracods from a lake which has simple hydrological characteristics. Lake Keilambete is a near-circular maar lake^{7,8} (area of lake 2.7 km²; area of catchment, that is, to crater rim = 4.2 km²) with input received only by direct precipitation, and water loss only by evaporation⁷. Thus, lake levels and the concentration of dissolved salts reflect changes in precipitation/evaporation, and documentation of sedimentary facies variations⁸ and palynology⁹, and identification of diagnostic ostracod species¹⁰ from cores spanning the past 10,000 yr have been used previously to infer changes in climate. The chronology of core KN (Fig. 1), representing 10,000 yr of sedimentation in 415 cm, is well controlled by 18 ¹⁴C dates^{7,9,11} that have been correlated from adjacent cores by comparison with a distinctive sequence of white aragonite and calcite microlaminar.

The Sr/Ca analyses of individual ostracods are presented in Fig. 1a. The $[\text{Sr/Ca}]_{\text{CaCO}_3}$ has been expressed as palaeosalinity using K_D (Sr/Ca) ~ 0.082 and the 1983 lake composition (mean salinity 73‰, and mean (Sr/Ca)_{CaCO₃} of 0.00635). This assumes that values for both salinity and Sr content are conservative and that the Ca content of the water is unimportant. *M. praenuncia* is generally present from 0 to 290 cm; ostracods are scarce in the interval 290–350 cm; and from 350 to 415 cm *A. robusta* is

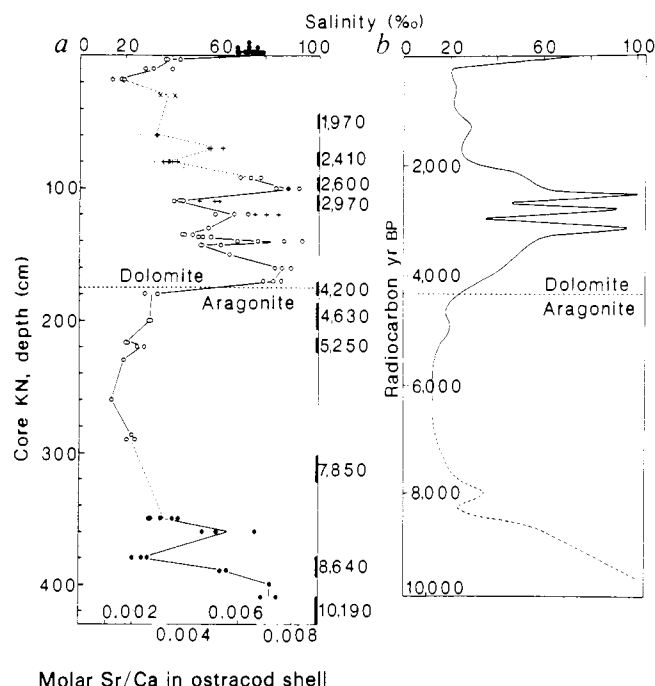


Fig. 1 *a*, Palaeosalinity curve for Lake Keilambete based on Sr/Ca composition (lower scale) for ostracods from 34 horizons within core KN. The data are principally from *Australocypris robusta* (●), *Mytilocypris praenuncia* (○), with some results for *Leptocythere lacustris* (×) and *Diacypris compacta* (+). Sr/Ca results for coexisting *M. praenuncia* and *D. compacta* at 110 and 120 cm suggest that *D. compacta* may incorporate more Sr ($K_D(\text{Sr/Ca}) \sim 0.10$) at the same conditions than does *M. praenuncia*. Between 20 and 90 cm, the dashed line records the Sr/Ca variations for *D. compacta* and *L. lacustris*. The parallel dotted line at lower salinity based on the species differences in $K_D(\text{Sr/Ca})$ may be a better indicator of salinity. Seventeen Sr/Ca values for modern *A. robusta* are incorporated in a histogram above zero depth. The horizontal dashed line at 175 cm indicates the first appearance of authigenic dolomite. *b*, Palaeosalinity curve (smoothed, based on 40 samples) derived from sediment grain-size analysis⁸. A calibration of the proportion of the >44- μm fraction of modern bottom muds as a function of present water depths (from 0 to 11 m) was applied to the proportion of the >44- μm fraction of samples from core K4.

present. At one horizon (100 cm) both species are recorded, and each has a similar Sr/Ca value. Analysis of up to five specimens from some levels show a tight cluster of Sr/Ca results. Between 20 and 90 cm, there is a paucity of *M. praenuncia* and we show the Sr/Ca results for *Leptocythere lacustris* and *Diacypris compacta*. We have insufficient information from modern lakes to establish $K_D(\text{Sr/Ca})$ for these species, but their analyses appear to fit within the trend described by analyses of the two principal species. The trend also compares favourably with Bowler's⁸ palaeosalinity curve interpreted from sedimentary grain-size analysis (Fig. 1*b*). We suggest that the $K_D(\text{Sr/Ca})$ for all these species may be similar.

There are several striking similarities between the palaeosalinity curves derived from $(\text{Sr/Ca})_{\text{ostracod}}$ and by grain-size analysis. The most saline water (at 2,600 yr BP, 100 cm) is estimated to have been ~100‰ by grain-size analysis and 90–95‰ by Sr/Ca. Similarly, the lowest salinity at 6,400 yr (260 cm) is estimated to have been 12 and 14‰ respectively. Both methods assume constancy of salts in the lake. One is based on fossil chemistry indicating past water chemistry, the other on texture of sediments to indicate past water levels, which in turn indicate palaeosalinity in a closed system. The close correspondence between the two independent sets of results is strong evidence for the correctness of the assumption. The significant difference in results from the two methods is that the Sr/Ca data suggest that a period of high salinity began earlier (at 4,200 yr, 175 cm) than that estimated by grain-size analysis. The interpretation based on Sr/Ca is logical, because at 175 cm and higher in the core, authigenic dolomite displaces authigenic aragonite. An abrupt increase in salinity (Mg content) at this point is likely. The previously documented salinity tolerances^{10,12} for *M. praenuncia* and *A. robusta* are 5–43‰ and 7–145‰, respectively. Our data suggest that *M. praenuncia* existed at up to 95‰ salinity.

Further comment on the palaeoclimatic implications of the Lake Keilambete region would only repeat Bowler's work^{7,8}. We conclude that the Sr/Ca of non-marine ostracods faithfully records palaeosalinity. Those species that have large salinity tolerances (for example 3–150‰) are exceedingly useful, and their Sr/Ca provides a potentially continuous palaeosalinometer, free of the discontinuities inherent in the estimation of palaeosalinity by recognition of biota with limited and specific salinity ranges. The reliability of this method will be governed by the hydrological setting of those lakes studied. Small tectonically stable closed basins seem suitable; volcanic and impact crater basins which have negligible groundwater

Table 1 Partial chemical composition of lake water and individual calcitic valves of the ostracod *Australocypris robusta* from Lake Keilambete at three different seasons

	Lake water					Individual ostracod valves				Distribution coefficient K_D (Sr/Ca)
	T(°C)	S(‰)	pH	Ca ²⁺ (p.p.m.)	Sr ²⁺ (p.p.m.)	Molar Sr/Ca	Ca (μg)	Sr (μg)	Molar Sr/Ca	
11 October 1982 (spring)	13.5	74	8.2	21.1	3.6	0.0780	33.64	0.4897	0.00666	0.0854
							39.73	0.5912	0.00681	0.0873
							41.13	0.5053	0.00562	0.0721
							44.05	0.6094	0.00633	0.0812
							53.38	0.7371	0.00632	0.0810
										Mean 0.0814 $\pm$ 0.006 (1 σ)
6 August 1983 (winter)	10.2	71.5	9.0	21.2	3.6	0.0777	2.87	0.0394	0.00628	0.0808
							44.57	0.6180	0.00634	0.0816
							52.52	0.6813	0.00593	0.0763
							56.72	0.7708	0.00622	0.0801
							59.24	0.7690	0.00594	0.0764
										Mean 0.0790 $\pm$ 0.003 (1 σ)
8 December 1983 (summer)	18.0	72.7	8.9	21.4	3.5	0.0748	16.74	0.2440	0.00667	0.0892
							22.21	0.2866	0.00590	0.0789
							34.50	0.4662	0.00618	0.0826
							39.94	0.5620	0.00644	0.0861
							41.06	0.6159	0.00686	0.0917
							45.98	0.6216	0.00618	0.0826
							55.19	0.7908	0.00655	0.0876
										Mean 0.0855 $\pm$ 0.004 (1 σ)

Salinity (S‰) equated to the total dissolved solutes by the formula of Williams¹⁴. Distribution coefficient, $K_D = [\text{Sr/Ca}]_{\text{CaCO}_3} / [\text{Sr/Ca}]_{\text{H}_2\text{O}}$. Chemical analyses^{5,6} were by very high-resolution inductively coupled argon plasma emission spectrometer (ICPAES). All concentrations were greater than an order of magnitude above the detection limits which are Ca²⁺ 0.02 p.p.b. (parts per 10⁹) and Sr²⁺ 0.03 p.p.b. in solution.

inflow or leakage are ideal. Because the varying $\delta^{18}\text{O}$ value of water during evaporation can be largely predicted as a function of salinity¹³, once palaeosalinity is determined from ostracod Sr/Ca, a semi-continuous record of calculated $\delta^{18}\text{O}$ of lake water might be coupled with ostracod Mg/Ca (ref. 5) or $\delta^{18}\text{O}$ of ostracod valves or other calcareous biota to indicate lacustrine palaeotemperatures.

At present, the interpretation of continental palaeoclimatic changes is largely restricted to consideration of changes in precipitation/evaporation (P/E), without indication of whether these variations are due to changes in precipitation (P), evaporation (E), or both. A resolution of quantitative continental palaeotemperature and lacustrine palaeosalinity would allow an independent estimation of past precipitation and temperature regimes.

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Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields

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The discovery of widely distributed populations of chroococcoid marine cyanobacteria (*Synechococcus* spp.) deep in the water column has focused attention on the autotrophic potential of small (<10 μm) size classes of marine phytoplankton^{1–5}. These organisms contain light-harvesting pigments which specifically absorb in the blue and green regions of the spectrum^{6–11}. Despite the fact that sea water acts as a monochromatic filter, allowing only the blue and blue-green wavelengths to penetrate¹², essentially all estimates of the photosynthetic rate of ultraphytoplankton at depth have ignored the changing spectral composition of the natural light field and simulated only the *in situ* light intensity^{4,5,13}. Here I show that the spectral composition of available light must be considered in estimates of water column productivity and that the depth of the euphotic zone varies between different ultraphytoplankton strains depending on the composition and organization of the photosynthetic apparatus.

Four experiments were performed, each modelled on the work of Jenkin¹⁴. In experiments 1–3, the photosynthetic performance of two phycoerythrin (PE)-containing *Synechococcus* clones was compared with that of PE-lacking *Synechococcus* clones at different light intensities in artificial white light (AWL) and at 15, 30,

45 and 60 m depths in the euphotic zone of clear ocean water (Table 1). In almost all cases, the cyanobacteria showed higher rates of photosynthesis in AWL than in natural light of comparable intensity, indicating that simulated *in situ* techniques probably overestimate the contribution of *Synechococcus* to primary productivity at depth. In AWL, the effect of decreasing light intensity was approximately equal for all the clones; the cellular rate of photosynthesis at 37 $\mu\text{E m}^{-2} \text{s}^{-1}$ was $\sim 20\%$ ($\pm 3\%$) of the rate at 260 $\mu\text{E m}^{-2} \text{s}^{-1}$. In contrast, the photosynthesis-irradiance relationships in natural light show marked differences between clones. In experiment 3, clone WH5701 showed no detectable ¹⁴C uptake at 60 m, although the two PE-containing clones, WH8018 and WH7803, showed photosynthesis rates of 6.78 and 8.42 fg C per cell per h, respectively. Thus, while the PE-lacking clone had reached its compensation light intensity, the rate of photosynthesis of clone WH8018 was decreased by only 70% compared with its rate at 15 m; the photosynthetic rate of clone WH7803 at 60 m was similarly decreased by only 67%. This pattern was repeated in all three experiments with the cyanobacteria; the photosynthetic rate at 60 m compared with that at 15 m was inhibited to the greatest extent in the PE-lacking clones, to a lesser extent in clone WH8018, and to the least extent in clone WH7803.

This rank order corresponds to the presence of accessory light-harvesting pigments which absorb light at wavelengths that penetrate to 60 m. The PE-lacking clones contain phycocyanin (PC) and allophycocyanin (APC) as their principal accessory pigments; these pigment proteins have absorption maxima at 620 and 650 nm, respectively¹⁵. Clone WH8018 contains PC and APC, but also contains a PE closely resembling C-phycoerythrin as the major light-harvesting pigment; this PE contains only phycoerythrobilin (PEB) chromophores (absorption maximum = 550 nm)^{7,16}. WH7803 also contains a PE as its major light-harvesting pigment; this PE, however, contains both PEB and phycocyanobilin (PUB) chromophores. The PUB chromophore has an absorption maximum at 492 nm and its presence enhances light-harvesting ability at blue wavelengths¹⁶.

The wavelength (λ) of maximum absorption *in vivo* and the presence or absence of different phycobiliproteins in marine *Synechococcus* spp. are under genetic control and independent of cell nutrient status¹⁷. Thus, the rank order for *in situ* photosynthesis observed in the present study should also be observed in natural populations. The relative differences between clones are probably not as great as would be seen in nature, as the cultured cells were grown in the presence of excess nutrients and undoubtedly contain more total pigment per cell than would individuals growing *in situ*. Increased total absorbance associated with high cellular pigment concentration would partially compensate for poorer relative absorption of blue light by genotypes which lack PE or contain a PE composed of only PEB chromophores.

Phycobiliproteins are closely related and are thought to have arisen by duplication of a common ancestral gene sequence^{18–22}. On the basis of subunit structure^{9,21}, sequence homology^{18–22}, distribution among taxa²², serological affinity²² and *in vivo* organization^{15,23,24}, the phycocyanins and allophycocyanins are viewed, phylogenetically, as the older forms^{18–22}. Phycoerythrins, particularly those containing both PEB and PUB chromophores, evolved more recently^{18–22}. In the marine *Synechococcus*, it seems that natural selection has favoured this evolutionary progression since, as shown here, each molecular step which increases the complexity of the photosynthetic apparatus, also increases the depth at which net photosynthesis can be achieved.

The theory that photosynthetic marine organisms possess pigments which complement the spectral composition of available light is an old one^{25,26} which has been widely accepted despite contrary empirical evidence for a number of species^{27,28}. The data presented here support the theory and suggest that complementary chromatic adaptation is an evolutionary rather than a physiological process in marine *Synechococcus* spp. If this is the case, the PE-containing genotypes, particularly those

Table 1 Comparison of photosynthetic rates of different clones

Experiment	Environmental conditions	Total PAR ($\mu\text{E m}^{-2} \text{s}^{-1}$)	% Surface irradiance	Rate of photosynthesis (fg C per cell per h)		
1	AWL	260	41.6	WH7803	WH8018	WH8007
	AWL	70	11.2	90.6	57.3	179.8
	AWL	36	5.8	41.8	23.1	84.4
	15 m	180	28.8	16.8	9.6	35.6
	30 m	90	14.4	60.5	35.1	41.3
	45 m	53	8.5	52.1	31.5	23.4
	60 m	33	5.3	39.9	12.6	18.7
2	AWL	260	33.3	15.8	8.5	2.0
	AWL	70	9.0	WH7803	WH8018	WH8007
	AWL	36	4.6	85.7	88.4	114.4
	15 m	220	28.2	42.3	44.7	50.9
	30 m	122	15.6	15.7	22.3	23.8
	45 m	70	9.0	40.2	55.6	46.8
	60 m	38	4.9	36.7	56.3	—
3	AWL	260	26.9	43.5	38.3	19.6
	AWL	70	7.3	26.2	19.4	9.2
	AWL	36	3.7	WH7803	WH8018	WH5701
	15 m	260	26.9	47.3	62.2	24.5
	30 m	115	11.9	—	28.7	24.9
	45 m	55	5.7	8.1	14.9	5.0
	60 m	28	2.9	20.4	27.7	7.3
4	AWL	260	43.3	19.5	24.4	5.2
	AWL	70	11.7	24.5	16.9	5.4
	AWL	36	6.0	6.8	8.4	ND
	20 m	240	40.0	WH7803	13-1	NEP
	40 m	100	16.7	29.6	3,451.3	1,181.9
	60 m	40	6.7	14.4	2,115.3	576.2
				5.0	725.4	190.8

Values are means of duplicate samples; ND indicates no detectable ^{14}C incorporation; dashes indicate lost samples; AWL, artificial white light. Clonal isolates of the eukaryotic ultraphytoplankton *Thalassiosira oceanica* Hasle and Heimdal (clone 13-1) and *Pavlova* sp. (clone NEP), and marine *Synechococcus* spp. clones WH7803 and WH8018 (which contain PE), and WH5701 and WH8018 (which lack PE) were obtained from the Center for Culture of Marine Phytoplankton (Bigelow Laboratory for Ocean Sciences). Clones 13-1 and NEP were isolated by R. R. L. Guillard in 1958 from surface waters in the Sargasso Sea and Gulf Stream, respectively. Clone WH8007 was isolated in 1980 from surface waters on the continental shelf of the northeastern United States by L. Provasoli. Isolation data for the remaining clones are given elsewhere⁷. The average diameter of the *Synechococcus* clones is 1–2 μm ; that of clone NEP is 6 μm ; and that of clone 13-1 is 3 μm . All experimental work was carried out onboard the R/V *Cape Hatteras* between 29 September and 14 October 1983, at four stations in the eastern edge of the Gulf Stream, between 29° and 34° N. Expendable bathythermograph data showed that the upper 75 m of the Gulf Stream in this region was isothermal at between 27.6 and 28.1 °C. Cultures were maintained in dilute cell suspensions (absorbance, $A_{\lambda \text{ max}} \approx 0.3\text{--}0.4$) at the early exponential phase of growth by repeated transfer into f/2-silica or f/2 media³⁶, and incubated at *in situ* temperatures (28 °C) with continuous illumination under Cool White fluorescent lights (General Electric) at an intensity of 50 $\mu\text{E m}^{-2} \text{s}^{-1}$. The cultures had been pre-acclimated to this light regime for 1 month (>10 generations) before the cruise. Before each experiment, the cultures were diluted into fresh sterile media as necessary to achieve approximately equal absorbance values ($A_{\lambda \text{ max}} \approx 0.2$), subsampled for cell counts, and spiked with ^{14}C -labelled sodium bicarbonate. The cultures were then subdivided into clear, 50-ml polycarbonate centrifuge tubes (Nalgene), sealed with a cap which allowed compensation for changes in external pressure, and incubated either onboard ship at 28 °C under Cool White fluorescent lights or at different depths in the water column in holders suspended from the ship's hydrowire. The holders were of clear Plexiglass, with clips holding the tubes to a bottom panel. Water circulated freely above and around the tubes. In the shipboard incubator, light was attenuated by neutral-density white Plexiglass panels. At the end of the incubation period, the rate of ^{14}C incorporation into organic material was determined by acidification and bubbling⁷; rates were corrected for dark uptake. Each experiment began approximately 1 h before local apparent noon, and continued for no more than 3 h to minimize the effect of diurnal changes in the spectral composition of solar radiation incident on the sea surface. Skies were partly cloudy during expt 1, and for the first half hour of expt 4, but were clear during expts 2 and 3 and during the last 2½ h of expt 4. The intensity of photosynthetically active radiation (PAR) and attenuation of light at 440, 520, 550 and 670 nm were determined using a Li-Cor underwater quantum meter and a four-channel underwater photometer. At all stations, the optical properties of the water corresponded to Jerlov type I oceanic water¹², with the 1% light level between 90 and 110 m; per cent transmittance in the top 10 m for light at 440, 520, 550 and 670 nm averaged 82.5, 79.8, 63.1 and 3.3%, respectively.

containing a phycoerythrin composed of PEB and PUB chromophores, would be expected to dominate natural populations of *Synechococcus* in oligotrophic waters where the photic zone can exceed 100 m; this appears to be the case as PE-lacking strains have never been found among oceanic isolates (J. B. Waterbury, personal communication) and strains containing a phycoerythrin with PEB and PUB chromophores are much more common among oceanic isolates than strains containing a phycoerythrin with only PEB chromophores^{6,7}. Note also that there is evidence for parallel evolution in *Oscillatoria erythraea* (Trichodesmium), the only other abundant planktonic cyanobacterium in oligotrophic ocean waters; this species also has a PE composed of both PEB and PUB chromophores²⁹.

In experiment 4, the performance of *Synechococcus* clone WH7803 was compared with that of two eukaryotic species: *Thalassiosira oceanica* Hasle and Heimdal (clone 13-1) and *Pavlova* sp. (clone NEP). The results (Table 1) show that while *Synechococcus* spp. containing a phycoerythrin with both PEB and PUB chromophores may have a competitive advantage in deep water compared with other cyanobacteria, the eukaryotes have a greater ability to adapt to the changing spectral composition of light fields in the open ocean. All three clones showed an approximately equal photosynthesis-irradiance relationship in AWL (Table 1), but when the rate of cellular photosynthesis at 60 m was compared with that at 15 m, the *Synechococcus* clone showed the greatest inhibition (Table 1); this is because the

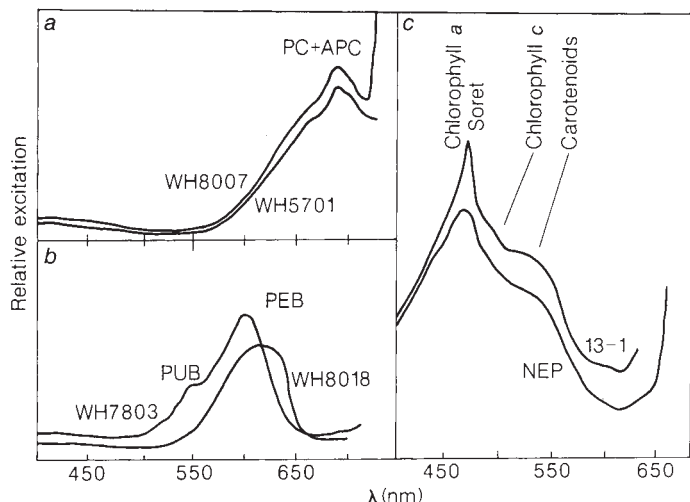


Fig. 1 *In vivo* excitation spectra for fluorescence emission by chlorophyll *a* at 680 nm in the marine *Synechococcus* (a, b) and eukaryotic ultraphytoplankton (c) clones used in the present study. Light energy absorbed by the Soret band of chlorophyll *a* is clearly transferred through the photosynthetic apparatus to a greater extent in the eukaryotes than in the cyanobacteria.

Methods. Cells from exponentially growing cultures were suspended on Whatman GF/B glass fibre filters and spectra obtained in ratio mode on a Baird Atomic SF-1 spectrofluorometer, with excitation and emission bandpass settings of 2 and 32 nm, respectively. Cells had been maintained at 24 °C under continuous illumination (Cool White fluorescent lights) at 50 $\mu\text{E m}^{-2} \text{s}^{-1}$.

Soret band of chlorophyll *a* can drive photosynthesis to a greater extent in the eukaryotes than the marine *Synechococcus* (Fig. 1). This significant difference in the organization of the photosynthetic apparatus of prokaryotic and eukaryotic ultraphytoplankton has not previously been reported and suggests strongly that eukaryotic ultraphytoplankton have the potential to out-compete marine *Synechococcus* at extreme depths where only blue and violet light penetrate, a conclusion which is independently supported by the work of H. W. Glover, M. Keller and R. R. L. Guillard (unpublished). In view of recent reports that small eukaryotic cells are occasionally more abundant than *Synechococcus* at depth³⁰⁻³⁵, eukaryotic ultraphytoplankton must be viewed as a potentially important source of new particulate material in deep ocean water.

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Odorant-sensitive adenylate cyclase may mediate olfactory reception

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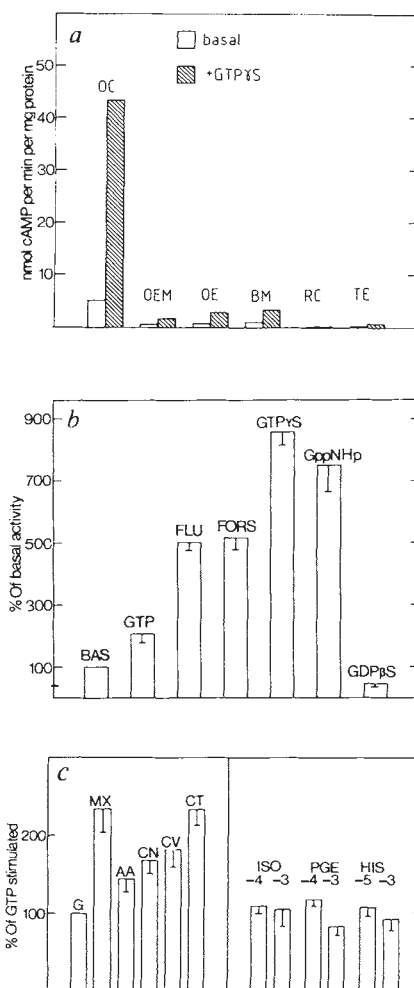
The mechanism of the sense of smell has long been a subject for theory and speculation¹⁻⁵. More recently, the notion of odorant recognition by stereospecific protein receptors has gained wide acceptance⁶⁻¹¹, but the receptor molecules remained elusive⁹⁻¹⁵. The recognition molecules are believed to be quite diverse^{9,11,13-15}, which would partly explain the unusual difficulties encountered in their isolation by conventional ligand-binding techniques^{12,13}. An alternative approach would be to probe the receptors through transducing components that may be common to all receptor types^{7-9,12-14}. Here we report the identification of one such transducing molecular component. This is an odorant-sensitive adenylate cyclase, present in very large concentrations in isolated dendritic membranes of olfactory sensory neurones. Odorant activation of the enzyme is ligand and tissue specific, and occurs only in the presence of GTP, suggesting the involvement of receptor(s) coupled to a guanine nucleotide binding protein (G-protein)¹⁵⁻¹⁹. The olfactory G-protein is independently identified by labelling with bacterial toxins, and found to be similar to stimulatory G-proteins in other systems¹⁷⁻¹⁹. Our results suggest a role for cyclic nucleotides in olfactory transduction^{13,20-22}, and point to a molecular analogy between olfaction and visual^{15,16}, hormone^{17,18} and neurotransmitter¹⁹ reception. Most importantly, the present findings reveal new ways to identify and isolate olfactory receptor proteins.

We have recently described a preparation of sensory cilia from frog olfactory neuroepithelium¹¹. These isolated extensions of the chemosensory dendritic membranes are believed to contain the olfactory receptive apparatus¹⁰⁻¹⁴, in much the same way as isolated retinal rod outer segments contain the molecular photoreceptive machinery^{15,16}. We found that the olfactory cilia preparation contains adenylate cyclase at nearly 15 times higher specific activity than membranes from brain or whole olfactory epithelium, both known as rich sources of the enzyme^{20,23} (Fig. 1a). From the change of specific activity after deciliation (Fig. 1a), we calculated that the cilia contain ~40% of the total epithelial adenylate cyclase, although they contain only ~3% of the total protein. Non-sensory cilia prepared from respiratory epithelium showed only 1% of the specific activity found in the olfactory organelles (Fig. 1a). GTP and its non-hydrolysable analogues guanosin 5'-(β , γ -imido)triphosphate (GppNHP) and guanosin 5'-O-(3-thio)triphosphate (GTP γ S), as well as fluoride ions and forskolin, enhanced the olfactory cilia enzymatic activity, while guanosin 5'-O-(2-thio)diphosphate (GDP β S), a GDP analogue, was inhibitory (Fig. 1b). Thus, the basic properties of olfactory adenylate cyclase are similar to those of hormone- and neurotransmitter-stimulated enzymes in other tissues¹⁷⁻¹⁹.

An important criterion for a functional role of adenylate

Fig. 1 *a*, Adenylate cyclase activity of frog olfactory cilia compared with that of membranes derived from other frog tissues. Open bars, basal activity levels; hatched bars, activity stimulated by 10 μ M GTP γ S (10 μ M GppNHp for turkey erythrocytes). OC, olfactory cilia preparation; OEM, membranes from deciliated olfactory epithelium; OE, membranes from whole olfactory epithelium; BM, membranes from brain; RC, respiratory cilia preparation; TE, turkey erythrocyte membranes. *b*, Activation of olfactory cilia adenylate cyclase by various effectors: BAS, basal activity; FORS, forskolin (10 μ M); GTP (10 μ M); FLU, sodium fluoride (10 mM); GppNHp (10 μ M); GTP γ S (10 μ M); GDP β S (1 μ M). *c*, Effect of odorants and other compounds on olfactory cilia adenylate cyclase in the presence of 10 μ M GTP. The results are normalized with respect to activity elicited by GTP alone (G), which is about twice the basal activity level; MX, equimolar mixture of the four indicated odorants (Aldrich) at 0.25 mM each: AA, *n*-amyl acetate; CN, 1,8-cineole; CV, L-carvone; CT, citral (a mixture of *cis* and *trans* isomers); each at 1 mM. ISO, isoprenaline (isoproterenol); PGE, prostaglandin-E₂; HIS, histamine, at log molar concentration as indicated. The lower concentration is in the appropriate physiological range, the higher is for comparison with the maximal odorant concentration used. Error bars in *b* and *c* (as in all following figures) represent standard deviation. Standard deviations in *a* are $\pm 15\%$ in any given preparation and up to $\pm 30\%$ between preparations. However, this larger error in absolute specific activities does not affect the activity ratios expressed in *b* and *c*.

Methods. Frogs (*Rana ridibunda*) were used for cilia preparation as described elsewhere^{10,11}. Briefly, epithelia were dissected and exposed to a 'calcium shock' by abruptly increasing Ca^{2+} from $<1 \mu\text{M}$ to 10 mM. Two steps of differential centrifugation yielded a preparation containing $>80\%$ cilia fragments and membrane vesicles derived from them. Membranes from various frog tissues were prepared by a simplified procedure in which tissue was polytron-homogenized (30 s, 0°C) in 20 mM Tris-HCl, pH 8.0, saturated with phenylmethylsulphonyl fluoride (PMSF). After removal of tissue and intact cells by centrifugation at 500g and 1,700g, respectively, membranes were precipitated at 20,000g. Turkey erythrocyte membranes were prepared as described previously³². Cilia and membranes were stored at -70°C or -180°C and aliquots frozen and thawed only once to keep adenylate cyclase activity intact. Adenylate cyclase was assayed as described previously³⁷ in the presence of 0.5 mM 3-isobutyl-1-methyl xanthine (IBMX). Protein content of samples was measured by the method of Bradford³⁸, with bovine serum albumin as a standard.



cyclase activity in olfaction would be its modulation by odorous compounds in a cell-free system²⁴. To maximize the probability of observing an effect, experiments were carried out with a mixture of four odorants (citral, L-carvone, 1,8-cineol and *n*-amyl acetate), all shown electrophysiologically to be detected by the frog olfactory system^{7,8,13,25-27}. This odorant mixture enhanced the adenylate cyclase activity of olfactory cilia in a dose-dependent manner (Fig. 2a). Activation was already significant at 2.5 μ M of individual odorants. In several independent experiments, the maximal activation achieved at 0.25 mM of individual odorants was to 4.6 ± 0.8 of the basal value (2.3 ± 0.4 of the GTP-stimulated level). Odorant stimulation was seen only in the presence of GTP, suggesting dynamic regulation requiring GTP hydrolysis, while GTP γ S stimulated the enzyme maximally, eliminating the odorant effect (Fig. 2a). This behaviour is similar to that of other receptor-modulated adenylate cyclase systems¹⁷⁻¹⁹.

The following findings constitute additional evidence for the specificity and physiological relevance of the *in vitro* odorant response. (1) Odorants did not activate adenylate cyclase in membranes from unrelated tissues, for example, frog brain (Fig. 2b) or frog liver (not shown). (2) Although the odorants, either in mixture or individually applied, enhanced adenylate cyclase activity of olfactory cilia to various degrees, compounds known to be stimulatory in other membranes are ineffective (Fig. 1c). (3) The maximal *in vitro* odorant effect occurred at concentrations of organic compounds 10–100 times lower than those required for nonspecific modulation of adenylate cyclase via changes in membrane fluidity²⁸. (4) The odorant concentrations necessary to stimulate adenylate cyclase *in vitro* (a cell-popula-

tion response) correlated well with those known to give measurable *in vivo* summated electrophysiological recordings. Such recordings are typically carried out at 10^{-2} – 10^0 vapour saturation²⁵⁻²⁷ for odorous compounds respectively having vapour pressures of 5–0.05 mm Hg (at 25°C)^{25-27,29}. With molar water/air partition coefficients being in the range 1–1,000 (ref. 30), the physiological aqueous odorant concentrations would be 3×10^{-6} – 3×10^{-3} M, in good agreement with our experiments. (5) The low value (<0.5) of Hill slope for the *in vitro* odorant response (Fig. 2a) agrees well with the slopes of electrophysiological and psychophysical dose-response curves (see ref. 27). As similar *in vitro* values were also obtained for a single odorant (amyl acetate, not shown), the low Hill slope probably arises from receptor heterogeneity^{9,11,13,14}, which would also explain why saturation is not observed within the concentration range examined. Our finding that diverse ligands stimulate ciliary adenylate cyclase is also consistent with such receptor heterogeneity.

Inherent problems in the application of classical receptor binding techniques to olfactory research^{12,13} include the lack of defined pharmacological profiles and specific antagonists for individual receptor types, and the absence of 'negative controls' (odorant homologues that do not cause activation). These problems may at least partially be related to receptor heterogeneity, and could be alleviated by the future development of olfactory neuronal cell lines possessing defined odorant specificity¹⁴. Measurements of odorant-stimulated adenylate cyclase could help in the study of such cells.

The dependence of olfactory adenylate cyclase activity on guanine nucleotides suggests that a signal-transducing G-protein

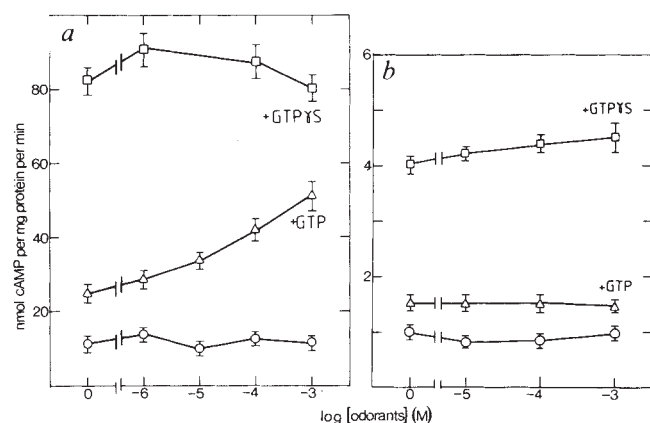


Fig. 2 Effect of odorants at increasing concentrations on adenylate cyclase activity in frog olfactory cilia (*a*) and frog brain membranes (*b*). Zero on abscissa means no odorants. GTP concentration is $10\ \mu\text{M}$ in both, GTP γS concentration is $10\ \mu\text{M}$ in brain membranes and $1\ \text{nM}$ in olfactory cilia. The lower curve in *a* and *b* is with no guanine nucleotides. Hill coefficients for the odorant response were calculated as the average slope of a graph of $\log Y/(1-Y)$ against $\log$ odorant concentration, where Y is the fraction of maximal stimulation attained. Maximal activation values from 55 to 80 nmol cyclic AMP per mg protein per min were used, to give a range of Hill coefficients of 0.35–0.49.

is involved, similar to the hormonal G-proteins^{17–19} and to visual transducin^{15,16}. To identify this protein, olfactory cilia were subjected to ^{32}P -ADP-ribosylation catalysed by bacterial toxins (Fig. 3). The cilia were found to contain a cholera toxin labelled polypeptide of relative molecular mass (M_r) 42,000 (42K) and a pertussis toxin labelled polypeptide of 40K. The first polypeptide has properties similar to those of the hormonal stimulatory G-protein (G_s), whereas the second is more like the inhibitory G-protein (G_i) or transducin^{15–19,31,32}. The polypeptide similar to G_s was found to be highly enriched in the cilia compared with membranes from deciliated epithelium, while the G_i -like protein is not (Fig. 3). This, together with the fact that odorants and GTP γS increase rather than decrease ciliary adenylate cyclase activity, suggest that the G_s -like protein is the specific neuronal membrane component responsible for receptor-cyclase coupling. The possible slight difference in M_r between the ciliary and liver G_s (Fig. 3), and the observation (Fig. 2a) that olfactory adenylate cyclase can be fully activated by GTP γS at much lower concentrations ($1\ \text{nM}$) than those necessary for its hormonal counterpart ($1\text{--}10\ \mu\text{M}$, ref. 32), may imply that the olfactory G-protein is a distinct G_s variant.

Additional evidence for the involvement of a G-protein in olfactory reception could be obtained from studies of the interaction between the ligand-activated receptor and the ADP-ribosylation site on the G-protein, as shown for visual and some hormone receptors^{33,34}. Our preliminary evidence (U.P. and D.L., unpublished) suggests that the toxin-catalysed modification of the ciliary G-protein synergizes with odorants in the activation of adenylate cyclase. More research is needed to resolve this issue.

The present study provides a direct identification of molecular components involved in olfactory reception. The enzyme adenylate cyclase is shown to be present in high amounts in the olfactory neuronal dendrites, and its activity is specifically enhanced by functional ligands in a cell-free system. These results, together with the previously reported *in vivo* modulation of olfactory responses by cyclic AMP analogues and phosphodiesterase inhibitors^{21,22}, provide all the criteria²⁴ necessary for establishing the role of cyclic AMP as a second messenger in olfactory reception. Measurements of odorant-stimulated adenylate cyclase in olfactory cilia thus provide the first *in vitro* assay for olfactory reactivity, and could complement electrophysiological recordings in the study of odorant recognition.

Our results suggest that the putative olfactory receptor molecules^{6–14} activate a cyclic nucleotide modulating enzyme

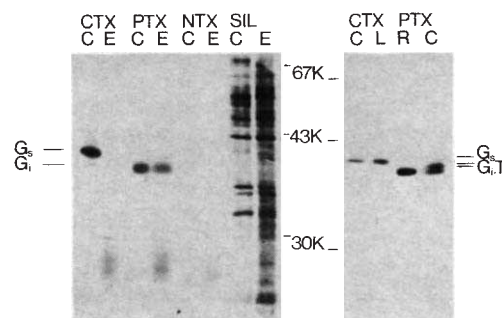


Fig. 3 SDS polyacrylamide gel electrophoresis autoradiography patterns of olfactory cilia (C), deciliated olfactory epithelial membranes (E), liver membranes (L) and retinal rod outer segments (R), all from frog, after ^{32}P -ADP-ribosylation in the absence (NTX) or presence of *Vibrio cholerae* toxin (CTX) or islet activating protein, *Bordetella pertussis* toxin (PTX) (both from List Biological Laboratories, Campbell, California). Silver staining patterns (SIL) are also shown, to indicate the relative amount of material used for labelling. ADP-ribosylation was carried out by a procedure similar to that of Schleifer *et al.*³⁹. Membranes ($200\ \mu\text{g ml}^{-1}$) were reacted at 37°C in $20\ \text{mM}$ Tris-HCl ($\text{pH } 7.6$) in the presence of $30\ \text{mM}$ thymidine, $1\ \text{mM}$ ATP, $0.1\ \text{mM}$ GTP, $5\ \text{mM}$ MgCl_2 , $1\ \text{mM}$ EDTA, $1\ \text{mM}$ dithiothreitol (DTT), $5\ \mu\text{M}$ ^{32}P -NAD $^+$ (NEN, $10\text{--}20\ \text{Ci mmol}^{-1}$), $3\ \text{mM}$ phosphoenolpyruvate, $5\ \text{U ml}^{-1}$ pyruvate kinase, 0.05% Triton X-100. The reaction was stopped after 1 h by adding electrophoresis sample buffer and boiling. The toxins ($10\ \mu\text{g ml}^{-1}$) were activated by preincubation with $20\ \text{mM}$ DTT at 37°C for 15 min. M_r standards ($\times 10^{-3}$) are marked; G_s , G_i , the positions of the heavy (α) chain of the stimulatory and inhibitory G-proteins; T, heavy (α) chain of visual transducin. The left part of the figure is a more heavily labelled autoradiogram showing that, for comparable amounts of silver-stained protein, the cilia contain much more cholera toxin substrate than do epithelial membranes, but both have similar amounts of pertussis toxin substrate. No labelling is seen in either preparation when toxins are absent. The right part of the figure is lightly loaded and exposed, and is intended to provide a more accurate definition of the labelled polypeptides. Olfactory cilia have a single cholera toxin polypeptide substrate, which seems to co-migrate with a similarly labelled polypeptide in frog liver. Both migrate at about 0.85 of the distance between the carbonic anhydrase (M_r 31K) and ovalbumin (43K) standards, similar to the major cholera toxin substrate found in most other tissues and species^{18,40}. This polypeptide is named 42 K or 45 K (we use the former) depending on the relative molecular mass assignment for the ovalbumin standard (43K or 45K–46K)⁴⁰. The olfactory preparations do not contain a 52K cholera toxin substrate, which is seen in some tissues and species and migrates more slowly than the ovalbumin standard⁴⁰. A weak band appearing below the 42K species in the leftmost lane may be a minor proteolytic cleavage product, and is seen occasionally in overloaded electrophoretograms. The pertussis toxin substrate of olfactory cilia is seen to be a doublet of polypeptide bands. The heavier component migrates between visual transducin and the G_s α -chains, and is putatively identified as the 40–41K polypeptide commonly ascribed to G_i α -chain^{18,40}. The lighter component almost co-migrates with transducin (39K). Multiple forms of pertussis toxin labelled polypeptides have been reported recently in nervous tissue^{41,42}.

via a GTP-binding signal-transducing protein. Olfaction thus appears to bear a striking molecular similarity to hormone, neurotransmitter and visual reception^{15–19}. This similarity can be used to clarify some aspects of olfactory function such as the role of transducing enzyme cascades in chemosensory signal amplification (see ref. 15). In parallel, olfaction may be viewed as a model for other molecular recognition processes. The fact that olfactory cilia preparations contain practically pure dendritic membranes derived from a single neuronal class, and are highly enriched in transducing components, make them suitable for studying general aspects of signal transfer in neuronal membranes.

The resemblance between olfaction and other transmembrane signalling mechanisms probably also applies to the receptor

molecules themselves. Thus, olfactory receptors may be integral membrane glycoproteins¹⁴ that interact with G-protein(s), as are the β -adrenergic receptor³⁵ or rhodopsin¹⁶. Such clues should be useful in devising new strategies for olfactory receptor studies. Accordingly, we are now examining the effect of lectins and monoclonal antibodies against defined ciliary surface glycoproteins^{11,36} on ciliary adenylate cyclase activity, and studying the direct association of glycoprotein receptor candidates with olfactory G-proteins.

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Vaccination with purified microgamete antigens prevents transmission of rodent malaria

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Malaria vaccination with preparations of microgametes has been shown to inhibit transmission of *Plasmodium* spp. to the mosquito vectors of avian^{1,2}, rodent³ and simian^{4,5} parasites. This transmission-blocking immunity results from the induction of microgamete-agglutinating antibodies in the vaccinated host which, when ingested in a mosquito blood meal, react with exflagellating microgametes in the midgut to prevent fertilization and oocyst development. Here we have passively transferred the immunity with gamete-specific monoclonal antibodies raised against the rodent malaria parasite *Plasmodium yoelii nigeriensis*⁶, and an IgG2a isotype monoclonal antibody from a hybridoma cell line, PYG-1, has been used to identify the target antigens on the gametes and to affinity-purify sufficient quantities of specific gamete antigen to facilitate vaccination studies. This transmission-blocking monoclonal antibody immunoprecipitated microgamete antigens of apparent relative molecular mass (M_r) 67K (67,000), 59K, 57K, 42K and 35K. Immunization of mice with these proteins before infection and mosquito feeding led to a 85-99.7% reduction in transmission to the mosquito vector; vaccination via intravenous or intramuscular routes was equally effective and did not require an adjuvant.

Gametocytes were purified from blood infected with *P. yoelii nigeriensis* by centrifugation through a discontinuous Percoll gradient⁷, washed and stimulated to undergo gametogenesis¹. After exflagellation, the soluble male gamete antigens were applied to an affinity column of transmission-blocking monoclonal antibody PYG.1 coupled to a Sepharose 4B matrix, washed through and the bound protein was eluted (see Fig. 1 legend).

Table 1 Vaccination with purified microgamete antigens prevents transmission of malaria

Group	Dose(μ g)	Route of administration	Mean no. oocysts per mosquito (range)	No. mosquitoes infected/total
1	40	i.v.	13 (0-60)	37/50
	40	i.m.	33 (0-120)	46/50
2	40, 30	i.v.	1 (0-7)	15/50
	40, 30	i.m.	2(0-14)	18/50
Controls	—	—	350	60/60

Two groups of female BALB/c mice were injected i.v. or i.m. with 40 μ g of affinity-purified gamete antigen (see Fig. 1 legend). Two more groups of mice were immunized i.v. or i.m. with 40 μ g of soluble gamete antigen, followed 2 weeks later by a second dose of 30 μ g administered via the same route as the priming dose. All mice were infected i.v. with 10^6 *P. y. nigeriensis*-infected erythrocytes 14 days after the final immunization. Three days after infection, batches of mosquitoes were fed for 30 min on each group of mice. Mosquitoes were maintained at a temperature of 24-27 °C for 1 week, before dissection and microscopic examination of their midguts for oocysts. All mosquitoes fed on control mice were heavily infected with oocysts; a random sample of 5 mosquitoes revealed a mean of 350 oocysts per mosquito.

SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 1) revealed that the monoclonal antibody immunopurified one major antigen of apparent M_r 42K and a series of minor proteins of M_r 67K, 50K, 57K and 35K. These proteins are specific to the sexual stage of the parasite; no bands were eluted from a PYG.1 column after application of solubilized asexual-stage parasites.

Female BALB/c mice were immunized as follows. Two groups of mice were each injected with a single 40- μ g dose of purified gamete antigen either intravenously (i.v.) or intramuscularly (i.m.), while two other groups of mice were immunized with 40 μ g of gamete antigen by the i.v. or i.m. route, followed 2 weeks later by a second dose of 30 μ g injected as per the priming dose.

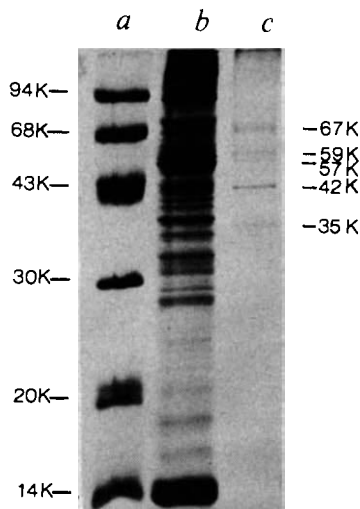


Fig. 1 SDS-PAGE analysis of *P. y. nigeriensis* gamete antigens. Lane a, M_r markers; lane b, total gamete antigen profile; lane c, PYG.1 affinity-purified gamete antigens.

Methods. Mouse blood with a gametocytaemia of 0.5–1.5% (parasitaemia 40–60%) was washed in suspended animation (SA) medium (0.21% Tris, 0.96% NaCl, 0.2% glucose, pH 7.4), in which gametogenesis is reversibly suppressed, and the gametocytes were purified by centrifugation through a discontinuous Percoll (Sigma) gradient as described in ref. 7. The gametocyte-enriched fraction was washed in SA medium and resuspended in gamete-releasing medium (2.5 vol. 5% NaCl, 2.5 vol. 10% glucose, 20 vol. 1.46% NaHCO_3 and 100 vol. heat-inactivated fetal calf serum adjusted to pH 8.0; ref. 1). After exflagellation, the male microgametes were separated by differential centrifugation and pelleted at 13,000g. Microgamete antigens were extracted on ice in 10 vol. of solubilizing medium (50 mM Tris pH 8.2 containing 1% Nonidet P-40, 5 mM EDTA, 5 mM EGTA, 5 mM iodoacetamide, 1 mM phenylmethylsulphonyl fluoride and 0.1 mM TLCK). Insoluble material was removed by centrifugation at 100,000g for 30 min at 4°C. The soluble gamete antigens were applied to an affinity column of transmission-blocking monoclonal antibody PYG.1 coupled to a Sepharose 4B matrix equilibrated with 10 mM Tris pH 8.0 containing 0.5 M NaCl and 5% (v/v) solubilizing medium. The column was washed with equilibrating buffer and the bound protein eluted with 50 mM diethylamine (pH 11.5) into tubes containing 1 M Tris pH 8.0. After neutralization, dialysis and concentration, the affinity-purified antigen was reappplied to the column and the affinity-purification procedure repeated. After further dialysis and concentration, the affinity-purified antigens were analysed by SDS-PAGE on 10% gels. Storage before use was at -30°C .

Sera taken from mice 10 days after a second i.v. or i.m. vaccination produced immunofluorescent staining patterns, using formalin-fixed whole gametes, which were indistinguishable from those produced by the monoclonal antibody PYG.1 (data not shown). Furthermore, using a combination of Western blot analysis and enzyme-linked immunosorbent assay techniques, these sera were found to react with a single gamete polypeptide of apparent M_r 42K, similar to the major protein in the affinity-purified gamete antigen preparation (Fig. 2), and thus confirming the immunogenicity of the affinity-purified antigen.

To determine whether immunization induced effective transmission-blocking immunity, all mice were injected i.v. with 10^6 *P. y. nigeriensis*-infected erythrocytes 14 days after the final immunization, and mosquitoes were fed on them 3 days later. The mosquitoes were maintained at $24\text{--}27^\circ\text{C}$ for a further 7 days before dissection and enumeration of oocysts in the midguts. The results (Table 1) show that immunization with a single dose of antigen led, respectively, to a 95% (i.v. route) and 85% (i.m.) reduction in oocyst numbers relative to mosquitoes fed on unvaccinated animals. Animals given two doses

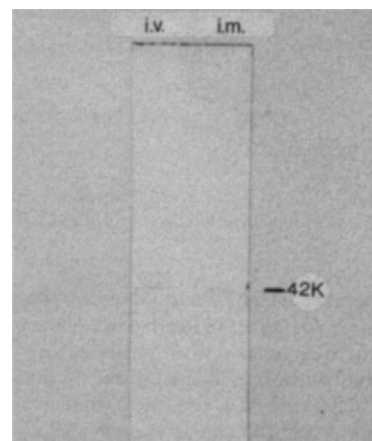


Fig. 2 Western blot analysis of *P. y. nigeriensis* gamete antigens. Solubilized unfractionated whole gamete proteins were separated by SDS-PAGE on a 10% gel and transferred electrophoretically to a nitrocellulose membrane. After blocking with 5% (w/v) bovine serum albumin in phosphate-buffered saline, the nitrocellulose membranes were incubated for 45 min with sera (diluted 1:100 in phosphate-buffered saline) taken from mice immunized i.v. or i.m. with affinity-purified gamete antigen. The membranes were washed and incubated with peroxidase-conjugated rabbit anti-mouse immunoglobulin for 45 min. The strips were then washed and stained with the substrate diaminobenzidine. The estimated M_r of the polypeptide is indicated.

of vaccine via the i.v. or i.m. routes were found to be almost totally uninfected to feeding mosquitoes, with oocyst numbers reduced by 99.7% and 99.3%, respectively. Indeed, more than two-thirds of mosquitoes in the latter groups were found to be uninfected. The transmission-blocking immunity was stage-specific in that no significant protection against the asexual erythrocytic forms of the disease was noted in any of the vaccinated animals (data not shown).

Although it is well documented that specific monoclonal antibodies directed against the sexual stages of *Plasmodium* will effectively block transmission of the parasite to its mosquito vector^{6,8–10}, this is the first report of purified soluble gamete antigens inducing an effective transmission-blocking immunity. The immunity is potent and does not require the co-administration of an adjuvant.

The mechanisms by which the host immune system mediates suppression of transmission appears to be serum-mediated (Fig. 2), as suggested by others^{2,6,10}. Microgametes are only susceptible to the action of the immune system immediately after exflagellation, and it is likely that the antibodies raised against the soluble antigen act to agglutinate gametes in the mosquito gut, preventing fertilization and consequently the transmission of infection.

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A uniform deleting element mediates the loss of κ genes in human B cells

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Human immunoglobulin light-chain genes become rearranged in an ordered fashion during pre-B-cell development such that rearrangement generally occurs in κ genes before λ genes (refs 1, 2). This ordered process includes an unanticipated deletion of the constant κ (C_κ) gene and κ enhancer sequence which precedes λ rearrangement¹⁻⁴, and the site of this deletional recombination was located 3' to the joining (J_κ) segments in 75% of cases studied. We have now characterized the recombinational element responsible for this event on three separate alleles and found them to be identical. This κ -deleting element recombined site-specifically with a palindromic signal (CACAGTG) located in the J_κ - C_κ intron. All losses of C_κ genes in other human B cells were mediated by this determinant, including the 25% of instances when this element recombined with sequences 5' to J_κ . In contrast, the κ -deleting element remained in its germline form on all successful κ -producing alleles. Moreover, κ loss is an evolutionarily conserved event, as the κ -deleting element appears to be the human homologue of the murine RS sequence⁵. Our results suggest that this element may help ensure isotypic and allelic exclusion of light chains and may be involved in the ordered use of human light-chain genes.

The two available light-chain classes in humans are used almost equally (κ , 60%; λ , 40%), yet the gene configuration of the excluded light-chain class in κ - and λ -producing B cells shows a marked dichotomy. Clonal cell lines or leukaemias of κ -producing B cells usually retain germline λ genes. In contrast, λ -producing B-cell lines have no remaining germline κ genes, at least the C_κ portion having usually been deleted³. Deletion of the κ genes is a normal developmental event unrelated to transformation⁶, and our examination of B-cell precursors indicated that this deletional event can occur before λ gene rearrangement^{1,2,4}. We further investigated the extent of the κ deletion and found that the C_κ region and the κ enhancer element

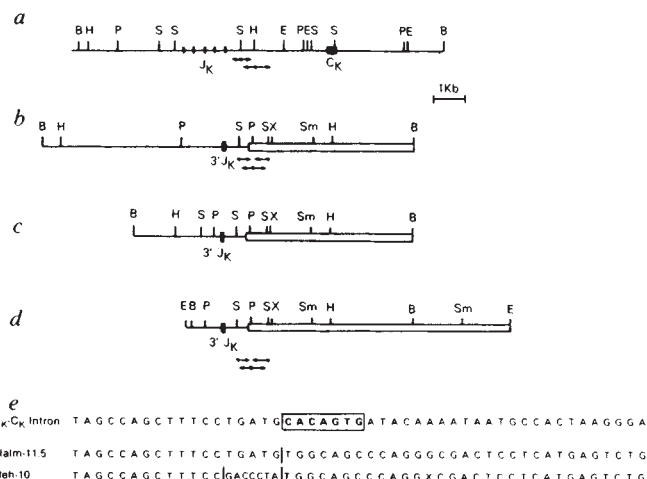


Fig. 2 Restriction map analysis, sequencing strategy and nucleotide sequence of the germline J_κ - C_κ region and three rearranged alleles bearing the 3' rearrangement. *a*, The germline J_κ - C_κ region restriction map in which arrows indicate sequencing strategy for M13 subclones sequenced by the Sanger dideoxy termination method²⁰. Three rearranged alleles (*b*, *c*, *d*) were cloned from Bam HI-digested (*b*, *c*) or Eco RI-digested (*d*) genomic DNA that was size-selected by preparative gel electrophoresis, ligated into the phage vectors Charon 28 or Charon 4A, and successfully screened with a J_κ segment probe^{25-27,31}. *b* is an 11.5-kb Bam HI J_κ -containing allele, *c* is an 8.8-kb Bam HI J_κ -containing allele from Nalm-6, while *d* is a 10-kb Eco RI J_κ -containing allele from Reh. In all three alleles a uniform κ -deleting element (κ de) has been introduced at their 3' end (open box), and all have undergone a separate rearrangement on the 5' side of J_κ . Nucleotide sequence analysis revealed the palindromic heptamer (CACAGTG) at 917 base pairs 3' to J_κ 5' in the germline J_κ - C_κ intron (*e*). The nucleotide sequence of the introduced κ de is identical in the Nalm-11.5 and Reh-10 alleles. Their recombination breakpoints are focused around the palindromic sequence. H, *Hind*III; B, *Bam*HI; P, *Pst*I; S, *Sac*I; E, *Eco*RI; X, *Xho*; Sm, *Sma*I.

were uniformly lost from the genome in all 20 instances examined (Fig. 1, Table 1). This was true for all deletions of κ genes in λ -bearing B cells, pre-B cells and those instances in which the excluded κ allele in κ -producing B cells was lost (Fig. 1, Table 1). All J_κ segments were also deleted in 5 of 20 instances, but were retained in a rearranged state in 15. Previous

Fig. 1 Deletions of J_κ and C_κ regions in human B cells. Representative genomic blots are presented of Bam HI-digested DNA which was electrophoresed in parallel on the same agarose gel, transferred to nitrocellulose paper, then hybridized with the indicated probes²⁵⁻²⁷. A human placenta served as a germline control, LY67 is a μ , λ -producing Burkitt lymphoma cell line²⁸, Nalm-6 and Reh are B-cell precursor acute lymphoblastic leukaemia cell lines² and SU-DHL-6 is a μ , κ -producing diffuse histiocytic lymphoma cell line²³. A 2.5-kb Eco RI genomic fragment was used as a C_κ probe and a 1.8-kb Sac I germline genomic fragment was used as a J_κ probe²⁹. Dash marks indicate the germline position and arrows denote rearrangements. A 0.7-kb Eco RI fragment containing the enhancer sequence revealed that the κ enhancer region was also deleted each time C_κ was lost (data not shown). Cytogenetic analysis of these cell lines revealed no translocations or obvious deletions of the short arm of chromosome 2. Asterisks indicate probes used in hybridization.

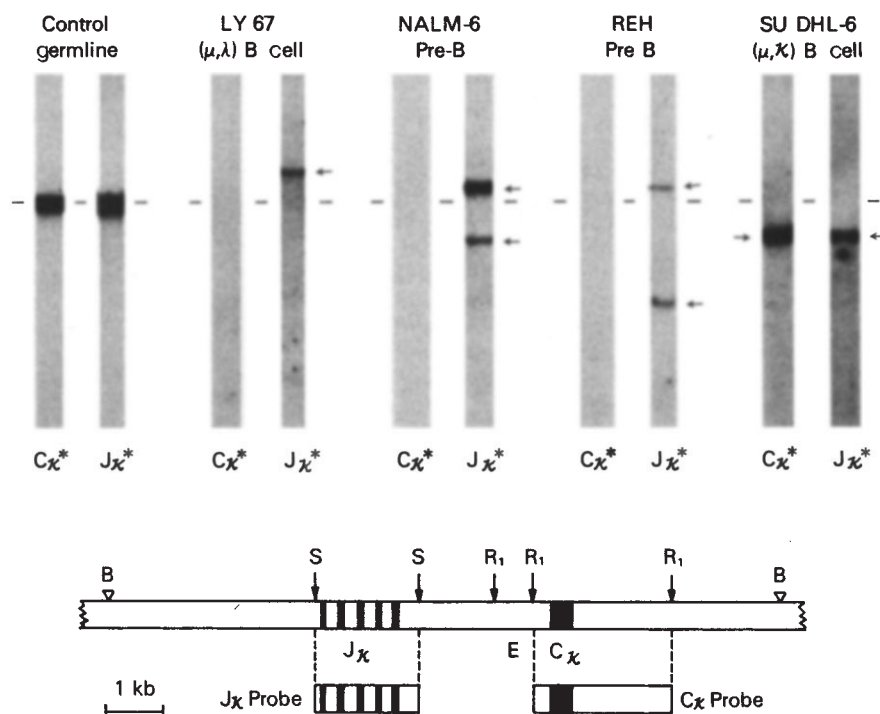


Table 1 The configuration of both κ gene alleles in 12 additional cell lines and leukaemias at different stages of B-cell development

Cell identification	Configuration of the κ alleles in		
	<i>Bam</i> HI genomic digests with probes of:		
Pre-B cells	$C_{\kappa}^*E_{\kappa}^*$	J_{κ}^*	κde^*
Nall-1	Deleted ($E_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)		Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)
ALL-RT-5		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Deleted ($J_{\kappa}-C_{\kappa}$)	Germline κde Rearranged (5' sequence/ κde)
RS 4; 11		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Deleted ($J_{\kappa}-C_{\kappa}$)	Germline κde Rearranged (5' sequence/ κde)
κ -Producing B cells			
CLL-RT		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Rearranged ($V/J_{\kappa}-C_{\kappa}$)	Germline κde Germline κde
SU-DHL-4		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Germline $J_{\kappa}-C_{\kappa}$	Germline κde Germline κde
BHM23		Rearranged ($V/J_{\kappa}-C_{\kappa}$)	Germline κde
SU-DHL-7	Deleted ($E_{\kappa}-C_{\kappa}$)	Rearranged ($V/J_{\kappa}-C_{\kappa}$) Germline $J_{\kappa}-C_{\kappa}$	Rearranged ($J_{\kappa}/\kappa de$) Germline κde Germline κde
λ -Producing B cells			
SU-DHL-5	Deleted ($E_{\kappa}-C_{\kappa}$)	Deleted ($J_{\kappa}-C_{\kappa}$)	Rearranged ($J_{\kappa}/\kappa de$) Rearranged (5' sequence/ κde)
RPMI 8392	Deleted ($E_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)		Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)
CLL-DG	Deleted ($E_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)		Rearranged ($J_{\kappa}/\kappa de$) Rearranged ($J_{\kappa}/\kappa de$)
Non-producing B cells			
SU-DHL-2		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Rearranged ($V/J_{\kappa}-C_{\kappa}$)	Germline κde Germline κde
VDS		Rearranged ($V/J_{\kappa}-C_{\kappa}$) Deleted ($E_{\kappa}-C_{\kappa}$)	Germline κde Rearranged ($J_{\kappa}/\kappa de$)

We examined three additional pre-B cells, Nall-1, ALL-RT-5 (ref. 2) and RS 4; 11 (ref. 22); four κ -producing B cells, CLL-RT, SU-DHL-4 (ref. 23), BHM23, SU-DHL-7 (ref. 23); three λ -producing B cells, SU-DHL-5 (ref. 23), RPMI 8392 (ref. 24) and CLL-DG; and two non-producing B-cell lines, SU-DHL-2 (ref. 23) and VDS. *Bam*HI-digested genomic DNA from individual cells was electrophoresed in parallel on the same gel and then hybridized to the previously described C_{κ}^* and J_{κ}^* probes, to a 0.7-kb *Eco*RI fragment containing the enhancer sequence (E_{κ}^*)¹⁷, and to the 2.5-kb *Hind*III/*Bam*HI κ -deleting element (κde^*) probe. Whenever the enhancer to C_{κ} ($E_{\kappa}-C_{\kappa}$) regions were deleted, the J_{κ} segments were found to be rearranged onto the same *Bam*HI fragment as the κde ($J_{\kappa}/\kappa de$). When the J_{κ} , E_{κ} and C_{κ} regions ($J_{\kappa}-C_{\kappa}$) were deleted, the κde was always rearranged to sequences apparently 5' to J_{κ} (5' sequence/ κde). In contrast all alleles with rearrangements limited to the 5' side of J_{κ} retained the E_{κ} and C_{κ} regions and were presumably attempts at V/J joining ($V/J_{\kappa}-C_{\kappa}$). These rearrangements always retained a germline κde .

studies indicated that one variable-region (V_{κ}) gene family was retained in its germline state³. We reasoned that cells in which the J_{κ} region was rearranged while the enhancer and C_{κ} region were deleted represented an opportunity to identify a 3' element that was being introduced by this deletional recombination. Therefore, we cloned the rearranged J_{κ} alleles from the B-cell precursor lines Nalm-6 and Reh.

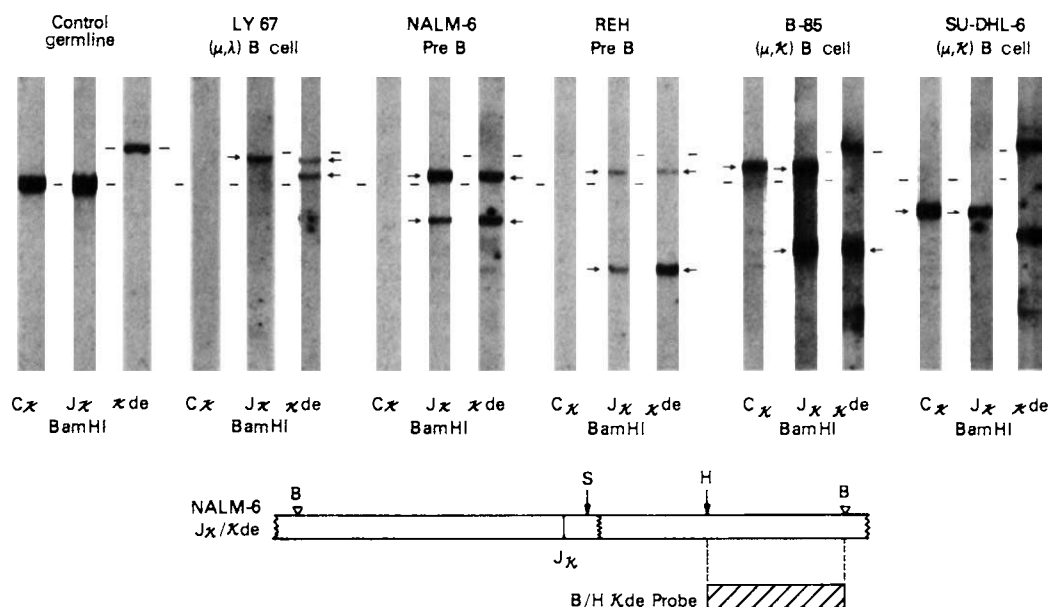
Both rearranged J_{κ} alleles from Nalm-6 and one rearranged J_{κ} allele from Reh were isolated from these pre-B-cell lines and compared with the germline $J_{\kappa}-C_{\kappa}$ region (Fig. 2). Restriction map analysis indicated that all three J_{κ} alleles had undergone a 5' rearrangement, presumably from attempts at V/J joining^{7,8}. Furthermore, all three J_{κ} alleles had undergone a 3' rearrangement which introduced a uniform element into the $J_{\kappa}-C_{\kappa}$ intron. DNA sequence analysis of the new 3' element from both cell lines confirmed that they were identical (Fig. 2). We term this introduced sequence the κ -deleting element (κde). As these three examples of a rearranged κde seemed to be recombining within a limited region of the $J_{\kappa}-C_{\kappa}$ intron (Fig. 2), we sequenced this previously uncharacterized portion of the germline $J_{\kappa}-C_{\kappa}$ intron, searching for a site-specific target for recombination. At 917 base pairs 3' to the last J_{κ} segment ($J_{\kappa}5$), we noted the palindromic heptanucleotide (CACAGTG). Interestingly, this is identical to the heptamer flanking germline V and J regions which is presumably involved in V/J joining⁹⁻¹¹. The Nalm-11.5 allele rearranged precisely at this palindrome and the Reh-10 allele rearranged at or near it (Fig. 2). This suggests that the introduction of the κde into the $J_{\kappa}-C_{\kappa}$ intron uses a site-specific recombination mechanism.

We found that this uniform κ -deleting element mediated the

loss of C_{κ} regions in all other instances that we examined. A 2.5-kilobase (kb) *Hind*III/*Bam*HI fragment prepared from the κde of Nalm-11.5 recognized a unique 14-kb *Bam*HI genomic fragment in its germline form (Fig. 3). The germline position of the deletional element is thus clearly separate from the germline $J_{\kappa}-C_{\kappa}$ *Bam*HI fragment. Figure 3 and Table 1 indicate that there is a rearrangement of this κde on every allele in which C_{κ} is deleted. In all instances in which C_{κ} is deleted but the J_{κ} segment is retained, the κde can be shown to co-occur with the same *Bam*HI restriction fragment with the J_{κ} segment. This is true not only in Nalm-6 and Reh, from which the κde was cloned, but also for 12 other alleles (Fig. 3, Table 1). Furthermore, one allele of the κde is always rearranged in those instances in which the J_{κ} as well as the enhancer and C_{κ} segment are deleted. One allele in LY67 and SU-DHL-6 demonstrate this in Fig. 3, and further examples are noted in Table 1. This indicates that the κde has a target site(s) 5' to J_{κ} as well as within the $J_{\kappa}-C_{\kappa}$ intron. In contrast, κ -producing cell lines always retain a germline configuration of the κde on the expressed allele, as illustrated by B-85, SU-DHL-6, CLL-RG, SU-DHL-4, BHM23 and SU-DHL-7 (Fig. 3, Table 1). In approximately 25% of cases, even κ -producing B cells display a deletion of the phenotypically excluded κ allele. In these cells, as shown by B-85, SU-DHL-6 and BHM23, the κde is also responsible for the elimination of κ genes. Thus, this same κde accounts for all losses of C_{κ} genes observed in pre-B cells, λ -producing B cells and κ -producing B cells.

Rearrangement and elimination of κ genes in λ -producing B cells is an evolutionarily conserved event^{5,12-14}. Even though the mouse expresses λ on only 5% of its B cells, the C_{κ} regions of

Fig. 3 Rearrangement of the κ -deleting element (κ de) in human B cells. Representative genomic blots of co-migrated *Bam*HI-digested DNA that was hybridized with the C_κ , J_κ and enhancer (E_κ) probes as well as a 2.5-kb *Hind*III/*Bam*HI fragment of the κ de (cross-hatched bars) from the 11.5-kb allele of Nalm-6. Dash marks indicate the germline position and arrows denote rearrangements. On alleles in which only the C_κ and E_κ regions have been deleted, the J_κ region co-occupies a *Bam*HI fragment with the rearranged κ de (LY67, Nalm-6, Reh). On alleles in which the entire J_κ - C_κ region is deleted, the κ de is also rearranged (LY67, SU-DHL-6). On the productive κ -producing allele of B-85 and SU-DHL-6, the J_κ and C_κ segment co-occupy a rearranged *Bam*HI fragment, but the κ de remains germline.



these cells have frequently been deleted. Durdik *et al.* have identified an element that recombines with the identical conserved palindrome (CACAGTG) within the J_κ - C_κ intron of the mouse⁵. This is the same intron heptamer that underwent a 5' rearrangement with a V_κ region in MPC11, resulting in a truncated κ -chain product^{15,16}. However, the major role of this signal appears to be as an acceptor site for the κ de, resulting in the removal of the C_κ and enhancer region. This heptamer also flanks germline V and J regions, suggesting that the recombinatorial enzymes that help activate this gene (V/J joining) also mediate its destruction.

The use of a κ -deleting element in two species suggests strongly that it has a functional role. By removing the κ possibility, the κ de might ensure λ gene utilization. Moreover, all κ de rearrangements result in the elimination of the κ enhancer element^{17,18}. Studies with a pSV2-CAT construct have suggested that only a limited quantity of a *trans*-acting factor recognizing that enhancer exists in host cells¹⁹. Thus, the deletion of the κ enhancer may transfer the effect of an immunoglobulin enhancer-specific factor to a putative enhancer within the λ locus^{20,21}. Alternatively, either the germline or rearranged κ de may itself encode a *trans*-acting factor that affects λ gene rearrangement or transcription. The κ -deleting element may therefore mediate the ordered use of light-chain genes and help ensure that B cells make only one light chain of a selected light-chain class.

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A molecular clone of HTLV-III with biological activity

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Acquired immune deficiency syndrome (AIDS) is an epidemic immunosuppressive disease characteristically associated with a depletion of T lymphocytes of the helper/inducer phenotype¹. Numerous converging lines of research have implicated a human T-cell lymphotropic retrovirus, HTLV-III, in the pathogenesis of AIDS²⁻⁵. Recently, several distinct forms of the HTLV-III genome were molecularly cloned in phage and extensively characterized^{6,7}. In the present study, a clone containing full-length HTLV-III proviral DNA⁷ was inserted into a plasmid and used to transfect cord blood T cells from normal newborn humans. We demonstrate that this molecular clone is infectious *in vitro* and causes marked cytopathic effects on T-cell cultures. This is the first direct evidence that the HTLV-III genome, rather than a minor component of the virus complex, is cytopathic for T cells. Using this biologically competent clone and mutants derived from it, it should now be possible to localize the subgenomic regions that contribute to the biological effects of HTLV-III.

Studies of cell-mediated immunity in AIDS victims reveal a generalized impairment of T-lymphocyte function accompanied by a reduction in T lymphocytes bearing the OKT4 antigen¹. The knowledge that a retrovirus (feline leukaemia virus) is often associated with severe immunodeficiency in cats⁸, and that the known human retroviruses are T-cell tropic and can impair immune function (see ref. 9 for a review), prompted speculation that a T-cell lymphotropic retrovirus might be the causal agent of AIDS. Two groups of human T-cell lymphotropic viruses,

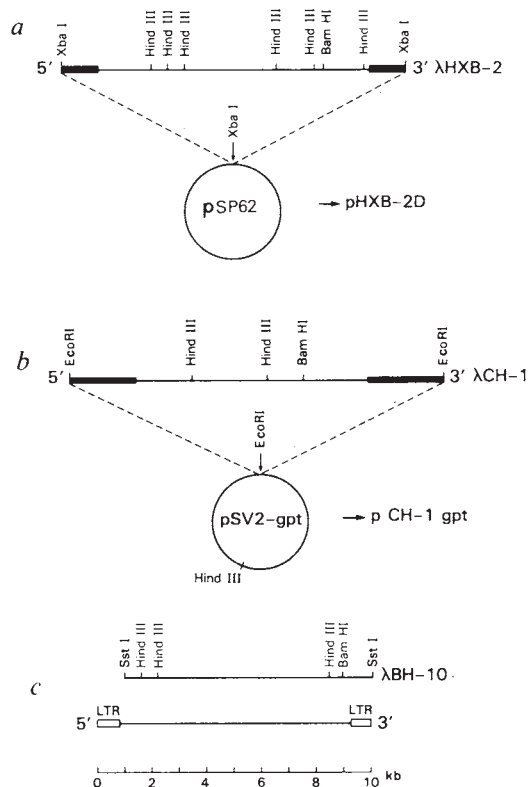


Fig. 1 Construction of plasmids containing HTLV-III or HTLV-I proviral sequences. A 12.7-kb Xba I fragment derived from λ HXB-2D, a molecular clone containing ~ 10 kb of HTLV-III proviral sequences, was inserted into the Xba I site in the polylinker of plasmid pSP62²⁸. Similarly, a 13.7-kb Eco RI fragment of λ CH-1 (a molecular clone containing ~ 9.0 kb of HTLV-I proviral sequences)²⁹ was inserted into the Eco RI site of pSV2gpt³⁰. These plasmid constructs were then transfected into DH-1 bacteria and used in protoplast fusion experiments. The thin horizontal line represents HTLV-III (a) or HTLV-I (b) sequences; the solid boxes represent flanking cellular sequences; and the open boxes in c represent the long terminal repeat (LTR) regions of the molecular clone λ BH-10. BH-10 is an incomplete viral clone of HTLV-III which lacks a 190-base pair segment in the 5'-LTR and leader sequence⁷. Insert from this clone was used to prepare the probe for hybridization analyses.

HTLV-I and HTLV-II, have been characterized and their association with T-cell malignancies reported^{10,11}. Recently, a third distinct group of retroviruses has been repeatedly isolated from patients with AIDS or from those at risk for the disease^{3,12,13}. These retroviruses, designated by different groups as human T-cell lymphotropic virus type III (HTLV-III)³, lymphadenopathy-associated virus (LAV)¹², and AIDS-related virus (ARV)¹³, are variants of the same virus as shown by DNA sequence analyses¹⁴. Our laboratory has shown that circulating antibody to HTLV-III can be detected in sera from over 90% of AIDS patients and from a high proportion of people in various high-risk groups, but not from sera of healthy people in non-risk groups⁵. These results, together with demonstrations that HTLV-III has a cytopathic effect on OKT4-positive (OKT4⁺) cells¹⁵, clearly implicate HTLV-III in the aetiology of AIDS.

HTLV-III has many properties in common with HTLV-I and HTLV-II, including a propensity to infect OKT4⁺ lymphocytes, a reverse transcriptase that prefers Mg^{2+} , a major core protein of relative molecular mass 23,000–25,000 and immunological relatedness to some of the major viral proteins⁴. In addition, these viruses have in common the property of *trans*-acting transcriptional activation, probably mediated by a unique viral gene (*lor*) product^{16,17}. However, HTLV-III differs strikingly from other HTLV members in at least two important aspects: (1) HTLV-III is highly replicative *in vivo* and shows considerable genomic diversity⁷, and (2) normal T cells infected with

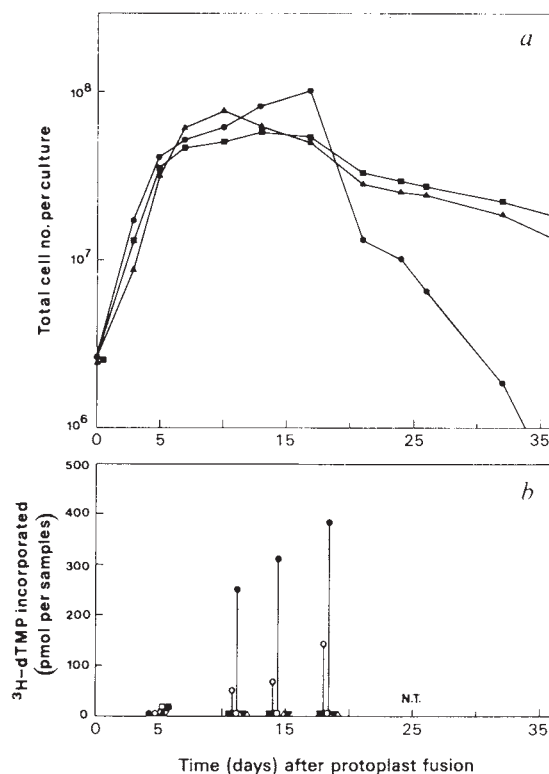


Fig. 2 Kinetics of cell growth and reverse transcriptase activity in cord blood mononuclear cell cultures following protoplast fusion. Mononuclear cells were prepared from cord blood samples using Ficoll Triosil and cultured for 5 days in media containing PHA. These cells were then fused with bacterial protoplasts carrying the plasmid pHXB-2D (○/●), pSV2neo (△/▲) or pCH-1gpt (□/■) and maintained in culture at a density of 5×10^5 cells ml^{-1} by addition of RPMI-1640 medium containing 20% fetal calf serum, 10% T-cell growth factor (interleukin-2) and antibiotics. Three parallel fusions using cells from different individuals were established for each plasmid. In a, the growth of the transfected cells is expressed as the mean value for three fusion experiments. Spent medium removed from two cultures at 5, 11, 14 and 18 days after fusion was concentrated 10-fold and assayed for the presence of reverse transcriptase using standard techniques³¹. The activity detected in each of the culture supernatants is expressed (b) as the amount of ³H-labelled deoxyribonucleoside monophosphate (³H-dTMP) incorporated (in pmol per 0.3-ml sample) using dT₁₅·(rA)_n as the template primer.

Methods. Protoplast fusion: Single colonies of bacteria bearing the plasmid of interest were grown in L-broth containing ampicillin to a density of $2-4 \times 10^8$ ml^{-1} and chloramphenicol was added to a final concentration of 250 μg per ml of broth. After incubation at 37°C for 2 h, gentamycin was added to 10 μg ml^{-1} and the cultures were re-incubated for 14–18 h to allow plasmid amplification. The bacteria were collected and resuspended in 2.5 ml of HEPES-buffered saline (HBS) pH 8.0, containing 20% sucrose. This suspension was placed on ice and at 5-min intervals, 0.8 ml of lysozyme solution (10 mg ml^{-1} in 0.25 M Tris-HCl pH 7.0), 1.0 ml of 0.25 M EDTA pH 8.0, and 1.0 ml of HBS were added sequentially. The suspension was incubated at 37°C for 10–30 min until >90% of the bacteria had formed protoplasts, then held on ice and slowly diluted to 50 ml with RPMI-1640 medium. For each fusion, 10^{10} bacterial protoplasts were combined with 2.5×10^6 PHA-stimulated core blood cells, washed and the pellet resuspended in 0.1 ml of RPMI-1640 medium. To these cells, 0.8 ml of polyethylene glycol fusion reagent (PEG-FR) was added dropwise over 1 min, the cells were left to stand for a further minute and then gradually diluted with RPMI-1640. PEG-FR was prepared as a 48% solution in RPMI-1640 medium and was pretreated by passing it over a resin bed²². After fusion, the cells were washed and returned to culture.

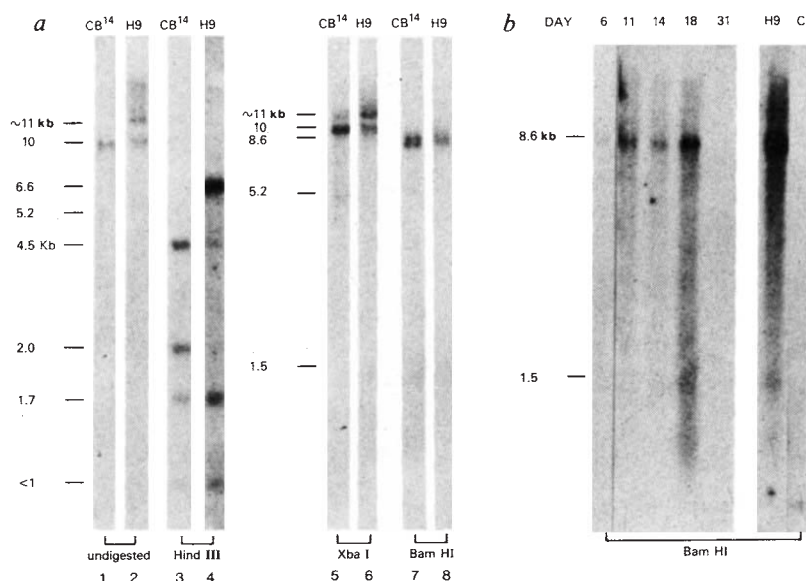


Fig. 3 Demonstration of the presence of HTLV-III sequences in cord blood cell cultures transfected with pHXB-2D. **a**, DNA of high relative molecular mass was isolated from H9/HTLV-III_B cells (H9) and from cord blood cell cultures 14 days after transfection with pHXB-2D (CB¹⁴) using standard protocols³². H9/HTLV-III_B is a permanent human T-cell line which continuously produces large quantities of HTLV-III. DNA (5 µg) was either left undigested (lanes 1 and 2) or digested separately with the enzymes *Hind*III (lanes 3, 4), *Xba*I (5, 6) or *Bam*HI (7, 8), electrophoresed in standard 0.8% agarose gels, blotted and hybridized to probe prepared from nick-translation of the BH-10 insert. The filters were washed in 0.5×SSC, 0.1% SDS at 65°C for 4–6 h before exposure. **b**, A time-course study in which DNA from cord blood cells transfected with pHXB-2D was analysed for HTLV-III sequences 6, 11, 14, 18 and 31 days after protoplast fusion. DNA (5 µg) was digested with *Bam*HI and hybridized to the BH-10 probe (under the conditions described for **a**). DNAs prepared from H9/HTLV-III (H9) and from PHA-stimulated cord blood cell cultures (C) were included as positive and negative controls, respectively.

HTLV-III degenerate rapidly in culture. In contrast, although HTLV-I and HTLV-II exert some cytopathic effects, they are able to efficiently immortalize some of the infected T cells in *in vitro* co-culture^{18–20} and in cell-free²¹ transmission studies. The mechanism by which HTLV abrogates normal T-cell function, leading to transformation (in the case of HTLV-I/ HTLV-II) or death (in the case of HTLV-III), is unknown.

In the present study, we used a transfection technique to investigate the biological properties of molecularly cloned HTLV-III DNA. The phage clone λHXB-2D, which contains full-length integrated provirus (~10 kilobases, kb) with cellular flanking sequences (12.7 kb total length), was recently isolated in our laboratory from an H9/HTLV-III genomic library constructed using *Xba*I⁷. We inserted the 12.7-kb *Xba*I fragment from this clone into plasmid pSP62 (Fig. 1) and introduced this construct into phytohaemagglutinin (PHA)-stimulated lymphocytes using the protoplast fusion technique²². Plasmids containing full-length HTLV-I ((pCH-Igpt) or no HTLV sequences (pSV2neo) were used as controls (Fig. 1).

Figure 2a summarizes the growth kinetics of T-cell cultures transfected with pHXB-2D, pSV2neo or pCH-Igpt. The growth of all cultures was comparable for the first 14 days after protoplast fusion. By day 18, however, the number of viable cells in cultures transfected with pHXB-2D had fallen dramatically: there was a 10-fold and a 100-fold reduction between days 18 and 21 and 18 and 32, respectively. Cultures transfected with either pSV2neo or pCH-Igpt showed only a 4–5-fold reduction over the same time period. When supernatant from the cultures was assayed for the presence of reverse transcriptase, activity was detected exclusively in cultures transfected with pHXB-2D (Fig. 2b). These data suggest that replicating virus was present in cultures 11–18 days after fusion with pHXB-2D protoplasts. The HTLV-I clone (pCH-Igpt) was apparently biologically inactive.

The presence of HTLV-III sequences in the transfected cells was demonstrated by Southern blot analysis (Fig. 3). DNA isolated from H9/HTLV-III_B was used as a positive control (H9). A 10-kb band, corresponding to unintegrated linear virus, was detected in undigested DNA samples prepared 14 days after transfection (Fig. 3a, lane 1). Digestion with *Xba*I revealed three distinct bands at 11, 10 and 5.2 kb; these co-migrate with bands frequently seen in undigested DNA from HTLV-III-infected H9 cells and other HTLV-III-infected cell lines and fresh tissues⁷ (Fig. 3a, lane 2). As *Xba*I does not cut within the viral sequences of clone pHXB-2D, these bands probably represent the nicked circular, linear and closed circular forms of unintegrated HTLV-III, respectively. In the *Xba*I-digested DNA samples, there was an increase in intensity of the nicked circular form compared with that in the undigested DNA sample (Fig.

3a, compare lanes 5 and 1); this was probably a result of nonspecific cleavage during incubation with the enzyme. The failure to demonstrate a DNA fragment of 12.7 kb shows that there was little or no plasmid carrying HTLV-III sequences in these cultures.

Digestion with *Hind*III, an enzyme which cuts the HTLV-III genome of pHXB-2D six times, yielded bands at 4.5, 2.0 (a doublet), 1.7 and 0.6 kb (a doublet) (Fig. 3a, lane 3), consistent with the fragments expected to be generated by *Hind*III digestion of linear unintegrated virus. This restriction pattern is clearly different from that of H9/HTLV-III_B (Fig. 3a, lane 4). The H9/HTLV-III_B cell line was derived from the human T-cell line HT, following co-culture with T lymphocytes obtained from several AIDS patients³, and contains many different HTLV-III forms. Thus, the differences in restriction patterns are consistent with H9/HTLV-III_B containing, as a minor component, a provirus equivalent to that of pHXB-2D. The major component of H9/HTLV-III differs, however, from the HTLV-III genome of pHXB-2D by the absence of a *Hind*III site ~3.5 kb from the 3' end of the virus⁷. *Bam*HI digestion of the DNA from the transfected cultures gave two fragments (8.6 and 1.5 kb) which result from a single cut of the linearized genome (Fig. 3a, lane 7). High-relative molecular mass 'smears' were not observed when DNA was digested with *Bam*HI. Therefore, we have no direct evidence that transfected HTLV-III DNA is integrated in the host cell genome. HTLV-III may integrate randomly and at too low a frequency to be detected by Southern blotting. Alternatively, it is conceivable that HTLV-III, like spleen necrosis virus and visna virus (cytopathic avian and ovine retroviruses), may not need to be integrated for virus production^{23,24}.

Expression of the HTLV-III *gag*-related proteins p15 and p24 by transfected cells was demonstrated using specific monoclonal antibodies (Fig. 4a–e): maximum expression was observed 18 days after transfection, when 4–11% and 5–9% of cells were reactive with antibody to p15 and p24, respectively (data not shown). Virus particles were detected by electron microscopy in all cultures 14–18 days after transfection with pHXB-2D (Fig. 4f). The particles contained condensed, truncated cores, which are characteristic of HTLV-III particles²⁵.

In time-course experiments (Fig. 3b), DNA isolated from a single culture 6, 11, 14, 18 and 31 days after transfection with pHXB-2D, was digested with *Bam*HI and analysed for HTLV-III sequences. Six days after transfection, an 8.6-kb DNA fragment was detected as a faint band; 18 days after transfection it was possible to detect a 1.5-kb DNA fragment in addition to the 8.6-kb fragment (Fig. 3b). The total amount of unintegrated virus in the cultures appeared to increase, as suggested by the increase in intensity of these bands with time; we interpret this as evidence that cells originally transfected with pHXB-2D are

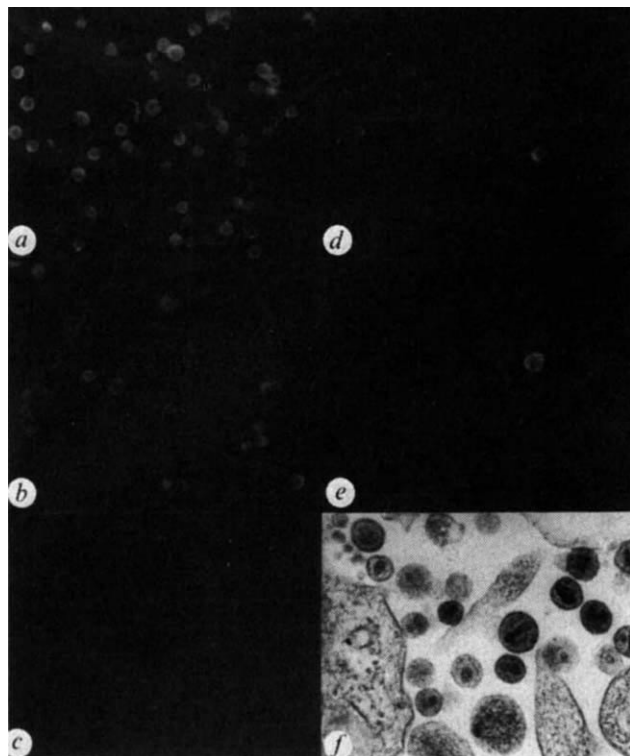


Fig. 4 Expression of HTLV-III p15 and p24 antigens and the production of virus particles by cord blood cell cultures transfected with pHXB-2D. Indirect immunofluorescence studies were performed using monoclonal antibodies against the HTLV-III gag-related proteins p24 (a, d) and p15 (b, e) (ref. 33 and A. Bodner, unpublished observations). Cells were fixed for 5 min using a 1:1 mixture of acetone/methanol and standard techniques. d, e, Cord blood cells removed from culture 18 days after transfection with pHXB-2D and stained to reveal the binding of these antibodies. A small percentage (4–11%) of the cells clearly express HTLV-III p24 and p15 antigens. In comparison, among H9/HTLV-III cultures (a, b), a much larger proportion of cells (70–90%) was positive for p24 and p15. c, Cord blood cells removed 18 days after transfection with pCH-Igpt (HTLV-I clone) were not labelled by these antibodies. f, Electron micrographs of cord blood cell cultures transfected with pHXB-2D show the presence of extracellular HTLV-III virus particles in cultures 18 days after protoplast fusion ($\times 45,000$).

able to produce fully infectious virus which is then transmitted within the culture.

No HTLV-III sequences were detected 31 days after transfection (Fig. 3b); at this point the culture may have contained only cells which failed to be infected by HTLV-III. This result is again consistent with the transfected DNA exerting a cytopathic effect on T cells. The finding that, at any stage, only a minor population of the transfected cells are apparently infected by the virus (<15% express viral proteins) suggests that the cytopathic effects may not result solely from direct viral infection and that secreted factors and/or other cell-to-cell interactions may play a part in the cytopathic phenomenon. To confirm this, we are presently using an *in situ* RNA hybridization technique to accurately assess the proportion of cells expressing viral messenger RNA transcripts (as this may be higher than the proportion expressing p15 or p24) (A.G.F. *et al.*, in preparation).

We have described the construction of a molecular clone of HTLV-III and its introduction into normal T cells, and demonstrated that T cells transfected with this clone contain unintegrated HTLV-III proviral sequences, express viral antigen and produce mature virus particles. Furthermore, we provide evidence that the virus produced from the HTLV-III genome is infectious and has marked cytopathic effects *in vitro*. Thus, HTLV-III is itself pathogenic for normal T cells. These results exclude the possibility that a minor component of the virus

complex is solely responsible for, or acts as a co-factor in, the cytopathic effects. The availability of this clone will allow us to elucidate the biological functions of the HTLV-III genome and, in particular, to localize the regions governing the cytopathic properties of the virus. In addition, as transfection allows the introduction of viral DNA into cells not normally infected by HTLV-III, we can use this system to investigate whether the tropism displayed by HTLV-III is determined exclusively at the level of the receptor^{26,27} or also at an intracellular level.

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Antibodies against metal chelates

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Because monoclonal antibodies can recognize and bind to specific groups of atoms such as tumour antigens, they have promise for use *in vivo* as carriers of radionuclides, drugs or other appended molecules for diagnosis and treatment of disease^{1–3}. Attachment of metal ions to antibodies by means of bifunctional chelating agents can add the diverse nuclear, physical and chemical properties of the metallic elements to these specific binding proteins (ref. 4 and refs therein). With the ultimate aim of engineering

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probe-binding properties into the antibodies themselves, we have now prepared monoclonal antibodies against the EDTA chelate of indium. These antibodies show a remarkable preference for indium chelates; changing to another metal such as scandium or gallium can decrease the antibody-binding constant by more than three orders of magnitude. These antibodies also introduce a new degree of control over the biological distributions of chelated radionuclides, markedly altering their uptake in tumours and normal organs.

Figure 1 shows the approximately 70 metallic elements that form stable EDTA chelates^{5,6}. These may possess properties such as radioactivity, luminescence or paramagnetism, leading to new molecular labels for use in nuclear medicine, immunoassay or magnetic resonance imaging^{2,3,7-10}. In solution, many of these chelates exist in dynamic equilibrium between several molecular configurations^{11,12}, but they can be crystallized as unique structures. Figure 2 shows a structure of iron(III)-EDTA; the EDTA chelates of indium(III), cadmium(II), cobalt(II) and manganese(II) crystallize in similar configurations¹³⁻¹⁷. Many other metal-EDTAs have different structures¹⁸⁻²³, thus, it is interesting to investigate whether an antibody against one metal-chelate can bind others in which the metal (or the chelator) is different.

To make antibodies against indium-EDTA, an antigen was prepared by conjugating L-SCN-C₆H₄-CH₂-EDTA to keyhole limpet haemocyanin using the methods of Meares *et al.*²⁴. After addition of indium(III), there was ~0.1 mg of attached chelate per mg of protein. To prepare antibody-producing hybridoma cell lines, spleen cells from BALB/c mice (multiply immunized with the antigen) were fused with a variant of the P3.653 myeloma cell line using the technique of Gerhard²⁵. The resulting hybridomas were screened by radioimmunoassay²⁶ for their ability to bind ¹¹¹In-L-NH₂-C₆H₄-CH₂-EDTA or ¹²⁵I-labelled bovine serum albumin bearing nonradioactive indium-EDTA groups; of 3,476 clones sampled, 102 produced antibodies possessing measurable affinity for the chelate. Based on their high titres and affinities, two IgG1 antibodies (from clones CHA255 and CHB235) were selected for further study. These monoclonal antibodies were purified from mouse ascites fluid

TABLE OF THE ELEMENTS																			
IA																			VIIIA
	IIA																	IIIA	IVA
	Be																	Al	
	Mg	IIIB	IVB	VB	VIB	VII	VIII	IB	II	III	IV	V	VI	VII	VIII	IX	X		
	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr		
	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Pb	Bi	Po	At		
	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At			
	Ra	Ac																	
Lanthanide Series		Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu				
Actinide Series		Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr				

Fig. 1 Metals that form stable EDTA chelates^{5,6}. Elements studied here are shown in bold face. A single underline indicates that the affinity of both antibodies for the metal-L-benzyl-EDTA chelate is 10–100 times less than for indium-L-benzyl-EDTA; a double underline 100–1,000 times less; a triple underline 1,000–10,000 times less.

by ion-exchange chromatography on DEAE-cellulose²⁷. Purity was routinely checked by SDS-polyacrylamide gel electrophoresis²⁸; for each purified sample, only one light-chain band and one heavy-chain band could be detected.

Binding constants for indium chelates were determined in triplicate by equilibrium dialysis and Scatchard analysis of a series of antibody-chelate solutions²⁹; as expected for IgG1, each antibody had 2 (±0.2) binding sites for chelate. Indium-EDTA labelled with ¹¹¹In or ¹⁴C was used in parallel experiments (with similar results), confirming that each antibody binds the chelator as well as the metal. The binding constants for metal chelates other than In-L-benzyl-EDTA were determined by equilibrium dialysis in competition with ¹¹¹In-L-benzyl-EDTA; this procedure was validated by independent determinations of the binding constants of indium-[¹⁴C]EDTA and ⁵⁷Co-L-benzyl-EDTA, and also by isotope dilution using non-radioactive In-L-benzyl-EDTA. ¹¹¹In-L-benzyl-EDTA did not bind detectably (<0.1%) to normal whole mouse IgG or to mouse serum.

Metal chelates selected for testing (Fig. 1) included trivalent ions that are similar to indium (Sc, Ga, Fe), and divalent ions whose EDTA chelates can have structures similar to that of indium-EDTA (Mn, Co, Cd). Inspection of Table 1a shows that changing the metal in the chelate can decrease the antibody-binding constant K_b , by more than three orders of magnitude. The metals whose chelates bind best are indium(III), iron(III) and cadmium(II); the available crystal structures for their chelates are all similar to that in Fig. 2¹³⁻¹⁷. Table 1 also shows that the K_b values are not well correlated with either ionic radii or metal-chelate stability constants.

Each antibody is much like the other with regard to selectivity for metals (Fig. 1); however, the antibodies differ markedly in their sensitivity to changes in the chelator. Table 1b shows that CHB235 is almost unaffected by whether the side chain is in the D- or L-configuration (see Fig. 2), or whether there is a side chain at all. CHA255 is rather sensitive to the presence of the L-benzyl side chain.

Because metal ions have affinity for amino-acid side chains bearing sulphur, nitrogen or oxygen^{5,6}, antibodies are fundamentally well suited to binding metal ions or metal chelates. Some cancer patients have been found to produce myeloma proteins (antibodies) that bind copper or calcium ions^{30,31}, and certain heavy metals such as platinum can be immunogenic³²⁻³⁴. The methods used here can be applied to prepare antibodies against a variety of metal chelates. It is immediately clear that particular metal chelates—and therefore particular metal ions—can be selectively bound, analysed and manipulated by immunochemical techniques^{2,3,35}. This novel connection of immunology to inorganic chemistry may find practical use; for example, in trace element analysis by immunoassay.

Table 1 Relative binding of metal chelates to monoclonal antibodies against an indium-EDTA analogue

a, Chelates of L-benzyl-EDTA with different metal ions				
Metals	r_{ion} (Å)	log K_s (EDTA)	K_b (CHA255)	K_b (CHB235)
Sc(III)	0.81	23.1	$3.0 \times 10^6 (\pm 13\%)$	$5 \times 10^4 (\pm 39\%)$
Fe(III)	0.64	25.1	$1.8 \times 10^8 (\pm 13\%)$	$2.4 \times 10^6 (\pm 18\%)$
Ga(III)	0.62	20.3	$5 \times 10^5 (\pm 45\%)$	$5.2 \times 10^4 (\pm 16\%)$
In(III)	0.81	25.0	$4.0 \times 10^9 (\pm 6.9\%)$	$1.1 \times 10^8 (\pm 7.9\%)$
Tb(III)	1.00	17.9	$1.2 \times 10^6 (\pm 12\%)$	$2.7 \times 10^4 (\pm 15\%)$
Yb(III)	0.94	19.5	$1.1 \times 10^6 (\pm 20\%)$	$2 \times 10^4 (\pm 90\%)$
Mn(II)	0.80	13.9	$2.8 \times 10^6 (\pm 9.1\%)$	$2.8 \times 10^4 (\pm 24\%)$
Co(II)	0.74	16.3	$2 \times 10^6 (\pm 37\%)$	$9.4 \times 10^4 (\pm 22\%)$
Co(III)	0.63	41.4	$8 \times 10^5 (\pm 60\%)$	$< 1 \times 10^4$
Cu(II)	0.69	18.8	$1.7 \times 10^6 (\pm 12\%)$	$8.1 \times 10^4 (\pm 11\%)$
Zn(II)	0.74	16.5	$1.4 \times 10^6 (\pm 14\%)$	$3.5 \times 10^4 (\pm 27\%)$
Cd(II)	0.97	16.5	$1.5 \times 10^7 (\pm 8.0\%)$	$1.6 \times 10^5 (\pm 8.1\%)$
b, Indium chelates with different chelators				
Chelators			K_b (CHA255)	K_b (CHB235)
L-Benzyl-EDTA			$4.0 \times 10^9 (\pm 6.9\%)$	$1.1 \times 10^8 (\pm 7.9\%)$
D-Benzyl-EDTA			$6 \times 10^7 (\pm 40\%)$	$3.6 \times 10^7 (\pm 9.3\%)$
EDTA			$1.7 \times 10^8 (\pm 8.3\%)$	$1.3 \times 10^8 (\pm 13\%)$
HED3A			$4.2 \times 10^7 (\pm 12\%)$	$4.0 \times 10^7 (\pm 14\%)$

a, Column 1, the metals whose L-benzyl-EDTA chelates (see Fig. 2) were tested against the indium chelate. Column 2, the ionic radius of each metal³⁰. Column 3, the logarithm of the stability constant^{5,6} for each metal-EDTA chelate at 25 °C. Columns 4 and 5, the antibody-binding constants of each metal-L-benzyl-EDTA chelate for the two different monoclonal antibodies studied (CHA255 and CHB235), in 0.05 M 2-hydroxyethyl piperazine ethane sulphonate, 0.1 M NaCl, 0.1% Na₂S₂O₃, 0.1% bovine serum albumin, pH 7.37 °C. Relative standard deviations are given in parentheses. For 'metal-free' Na⁺ L-benzyl-EDTA, K_b (CHA255) is $< 10^6$; in this case it is difficult to measure K_b accurately, as trace metal contaminants bind to the chelator. b, Column 1, the different chelators used to make indium chelates (see Fig. 2).

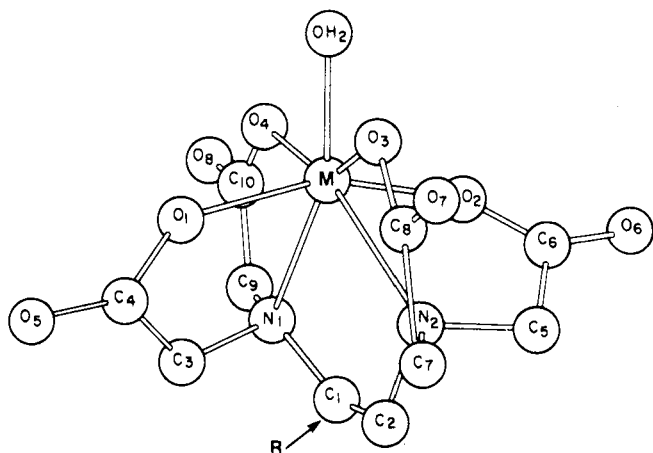


Fig. 2 Crystal structure of the Fe(III)-EDTA chelate, which is similar to those of In(III), Cd(II), Mn(II) and Co(II)¹³⁻¹⁷. EDTA supplies two nitrogen (N1, N2) and four oxygen (O1-O4) ligands to the metal (M), and a water molecule (OH₂) is also coordinated to the metal. For many of the experiments described here, a benzyl side chain (R = C₆H₅CH₂—) was attached to carbon-1, as indicated by the arrow (both the D- and L-configurations were studied). The chelator HED3A has an alcohol in place of one carboxylate group (for example, by replacement of oxygen-5 with two hydrogens). Drawing adapted from Hoard *et al.*¹⁴.

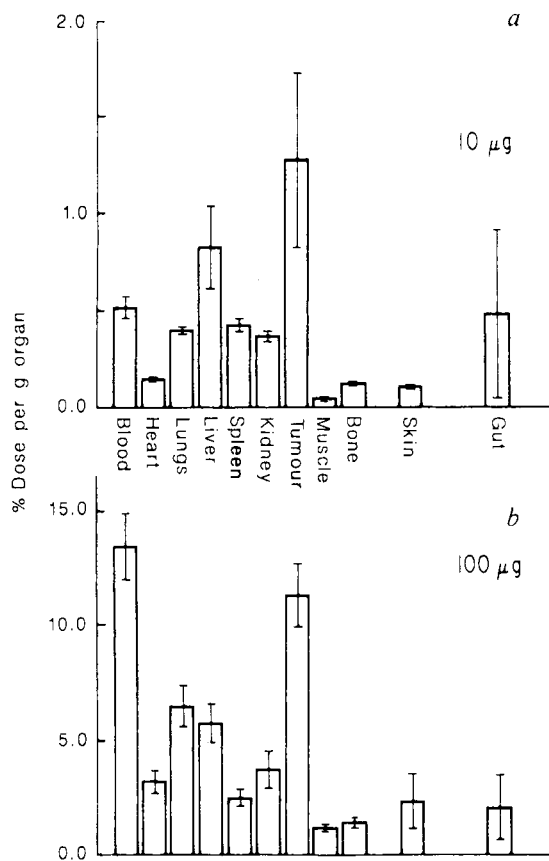


Fig. 3 Comparison of the distributions of radioactivity in BALB/c mice bearing KHJJ tumours³⁶, 24 h after injection of either 10 µg (a) or 100 µg (b) of ¹¹¹In-L-benzyl-EDTA plus a trace of ¹¹¹In-L-benzyl-EDTA. Error bars represent 1 s.d. Note that the range of concentrations in b is 8.5 times that in a. Similar results were obtained with the CHB235 antibody, but larger amounts were required. When ¹¹¹In-L-benzyl-EDTA was injected without anti-chelate antibody, the concentrations of radioactivity were all less than 0.05% per g; this is insignificant on the scale of a.

These antibodies are also capable of altering the biological properties of metal chelates. For example, consider their effect on the behaviour of ¹¹¹In-L-benzyl-EDTA in BALB/c mice. Intravenous injection³⁶ of a trace of the radiochelate together with 10 µg of CHA255 per mouse increases the biological half-life of the chelate from 40 ± 15 min to 6 ± 1 h; when the amount of antibody is increased to 100 µg, the half-life increases to 54 ± 15 h. Nonspecific antibody, or quantities of specific antibody smaller than 1 µg per mouse, have no measurable effect on the biological behaviour of the indium chelate.

These 'reversibly radiolabelled' antibodies behave in a way that is qualitatively different from covalently radiolabelled antibodies. Figure 3 shows tumour and organ concentrations of radioactivity 24 h after injection of ¹¹¹In-L-benzyl-EDTA and anti-chelate antibody in mice bearing KHJJ tumours. By varying the antibody concentration *in vivo*, it is possible to enhance the uptake of radioactivity in the tumour relative to the rest of the body (Fig. 3a). All the radioactivity concentrations are strikingly increased in the presence of the antibody, with the tumour concentration rising from <0.05% per g up to 11.3% per g (Fig. 3b). Clearly, anti-chelate antibodies can introduce a new degree of control over the biological distribution of chelated radionuclides and other chelates used in medical diagnosis and therapy.

As the antibodies have no known affinity for the tumour, they are presumed to accumulate passively³⁷. Extension of the present work to prepare monoclonal antibodies with dual antigen specificity^{38,39}, which could simultaneously bind a metal chelate and a physiological antigen, may provide further improvements.

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Active γ -carboxylated human factor IX expressed using recombinant DNA techniques

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Factor IX (Christmas factor), a vitamin K-dependent plasma protein made in the liver, functions in the middle phase of the intrinsic pathway of blood coagulation¹. A functional deficiency of factor IX underlies haemophilia B, a chromosome X-linked recessive disease for which the major therapeutic approach is replacement treatment using factor IX concentrates. The cloning and characterization of the gene for human factor IX²⁻⁴ would mean that human factor IX could be produced in greater yield and purity through using recombinant DNA techniques⁵. We have now used a human factor IX cDNA clone⁴, inserted into a vaccinia virus-derived vector, to infect human hepatoma cells which normally produce no factor IX⁶, and mouse fibroblasts. Fully active factor IX was produced by the hepatoma cells, whereas the fibroblasts produced a protein less active than natural factor IX, even in the presence of high levels of vitamin K. Human factor IX is extensively post-translationally modified, and thus represents probably the most complex protein produced in active form by recombinant DNA techniques to date. Our study also illustrates the potential of vaccinia virus-based vectors for expressing significant amounts of complex, clinically useful proteins in eukaryotic cells, in addition to its already demonstrated usefulness for producing live recombinant vaccines⁷⁻⁹.

Factor IX is a protein of extraordinary complexity. Several sialic carbohydrates are linked to the protein chain and experiments have shown that these groups may be important for the activity of the molecule¹⁰. The first 12 glutamic acid residues of the mature enzyme are converted to γ -carboxyglutamic acids in a vitamin K-dependent process. These modifications are essential for the activation of factor IX. In addition, one aspartic acid residue (position 64) is hydroxylated¹¹⁻¹³, and may also be important for activation of the molecule^{11,12,14}.

Activation of factor IX from its zymogen form occurs through the action of activated factor XI. It involves two specific proteolytic cleavages, and releases an activation peptide, and the active proteolytic enzyme, factor IXa, whose two chains are held together by disulphide bridges. In addition, analysis of the factor IX gene sequence shows that the primary translation product of factor IX messenger RNA is a pre-pro form^{3,4}, though the zymogen precursor has not yet been characterized.

In a search for cells which can produce biologically active factor IX, we attempted to express the protein in several different cell lines. We first looked at a human hepatoma cell line, HepG2 (ref. 6), which synthesizes many different plasma proteins,

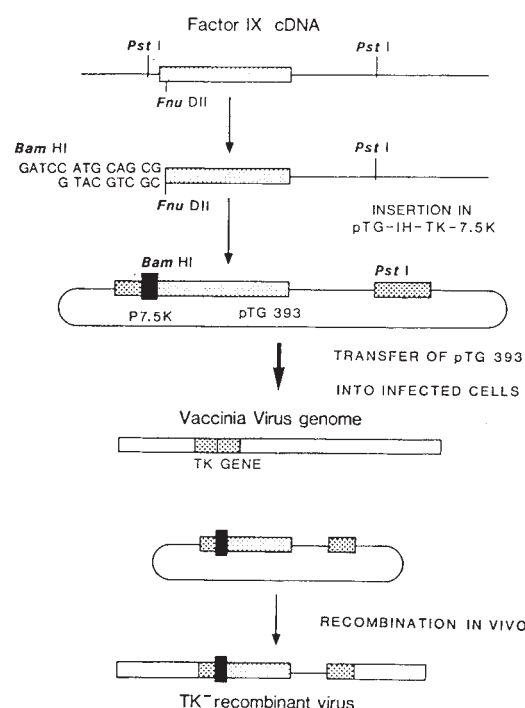


Fig. 1 Insertion of the factor IX cDNA coding sequence into vaccinia virus. The *Fnu*DII/*Pst*I fragment was isolated from the factor IX cDNA clone⁴, and the coding sequence at the 5' end together with a *Bam*HI site cohesive end were restored using a synthetic DNA linker. This fragment was then transferred to the plasmid pTG1H-tk-P7.5K (ref. 9) which contains the vaccinia virus *tk* gene insertionally inactivated by the presence of the early 7.5K promoter of vaccinia virus. The resulting plasmid, pTG393, contains the factor IX cDNA sequence immediately adjacent to the 7.5K promoter, and flanked on either side by parts of the vaccinia *tk* gene. These flanking regions allowed the *in vivo* recombination of the factor IX sequence of pTG393, together with the 7.5K promoter, into the N7 strain of vaccinia virus (derived from the Copenhagen strain) using procedures already described⁹. The fine dotted regions represent the factor IX cDNA sequence, the larger dotted regions the vaccinia *tk* gene, and the black area the 7.5K promoter.

including factor X and protein C, which are closely related to factor IX and undergo many of the same modifications. However, no factor IX synthesis was found in these cells⁶, nor have we been able to detect significant levels of factor IX mRNA in the cells (unpublished observations).

To express factor IX in HepG2 cells, we used vaccinia virus as a vector. The strategy for insertion of a gene into the vaccinia genome has been described previously⁷⁻⁹ and is based on a homologous recombination between the thymidine kinase (TK) gene in the viral genome and the plasmid bearing the gene to be expressed, generating a TK⁻ recombinant virus (Fig. 1). One such TK⁻ virus was analysed by Southern blotting to verify that the pattern of restriction fragments of the cDNA inserted in the vaccinia genome was the same as in the starting plasmid (Fig. 2).

To establish whether the inserted factor IX gene was expressed during vaccinia infection, HepG2 cells infected for 15 h were labelled for 5 h with ³⁵S-methionine. Both cell extracts and culture medium were immunoprecipitated with IgGs prepared from rabbit anti-human factor IX antiserum. Two relatively diffuse bands, of apparent relative molecular masses (*M*_s) 79,000 (79K) and 71K, were specifically immunoprecipitated from the medium of HepG2 cells infected with the factor IX recombinant vaccinia virus (Fig. 3A, lane 2) but were absent from cells infected with wild-type virus (lane 1) or from media precipitated with non-immune serum (lanes 5-8). The smaller of the specifically immunoprecipitated bands has an apparent

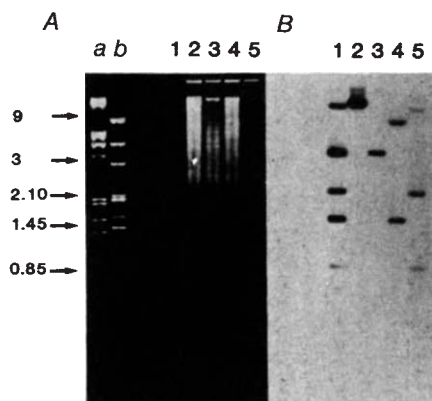


Fig. 2 Verification of the insertion of the factor IX cDNA sequence into vaccinia virus. **A**, Ethidium bromide-stained gel before transfer. Lane *a*, M_r standards from a digest of λ DNA with *Eco*RI and *Hind*III; lane *b*, a mixture of different digests of the plasmid, pTG393, carrying the factor IX sequence within the vaccinia *tk* gene. A different digest, with *Pst*I alone, *Pst*I + *Hind*III, *Pst*I + *Ava*I and *Pst*I + *Ava*II were mixed before electrophoresis. The fragments which should contain factor IX sequences are marked by arrows, and have sizes of 9.0, 3.0, 2.11, 1.45 and 0.85 kilobases, respectively. Lane 1, mixture of pTG393 digests as for lane *b*, but with 10 ng of DNA instead of 1.0 μ g; lanes 2–5, DNA of infected cells digested with *Pst*I (lane 2), *Pst*I + *Hind*III (3), *Pst*I + *Ava*I (4), *Pst*I + *Ava*II (5). **B**, Autoradiogram of nitrocellulose filter hybridized with 32 P-labelled factor IX probe. Lanes 1–5 correspond to the same lanes as for **A**. The two largest bands in lanes 4 and 5 (arrows on right) correspond to end fragments of the factor IX portion of pTG393 flanked by vaccinia sequence, and so do not correspond in size to the hybridizing fragments of pTG393 alone (lane 1).

Methods. A 75-cm² flask of confluent chick embryo primary fibroblast cells was infected with 0.5 plaque-forming units per cell of recombinant vaccinia virus. After 2 days, cells were collected, lysed and DNA was prepared by standard procedures. The DNA was digested with various restriction enzymes, electrophoresed on a 1% agarose gel and blotted onto nitrocellulose. The filter was hybridized with 32 P-labelled factor IX cDNA probe.

size consistent with that of natural factor IX; the other may represent a precursor form of factor IX, or perhaps a molecule containing a different form of glycosylation.

The analysis of supernatants from HepG2 cells infected and labelled in the presence of tunicamycin, an inhibitor of N-glycosylation, revealed several specific bands of greater mobility from cells infected with the recombinant vaccinia virus (Fig. 3A, lane 3). Moreover, the ability of unlabelled factor IX to compete with the medium polypeptides (Fig. 3B, lanes 1, 3) provided additional evidence that the immunoprecipitated polypeptides were factor IX molecules.

To characterize further recombinant factor IX produced by the vaccinia vector, and to determine whether the product exhibited biological activity, medium from infected cells was fractionated using barium citrate¹⁵. This fractionation is based on the ability of γ -carboxylated proteins to adsorb to the insoluble salt of barium citrate¹⁵. Fully γ -carboxylated molecules co-precipitate with the salt, while non-carboxylated molecules stay in solution. The properties of a partially modified protein are not known in the case of factor IX, but studies with prothrombin, another γ -carboxylated protein, have shown that the extent of precipitation is related to the number of γ -carboxyglutamic acid residues present¹⁵. We used a commercially available enzyme-linked immunosorbent assay to quantitate factor IX antigen through the fractionation procedure, and the activity of barium citrate-adsorbed fractions was measured as their ability to restore coagulability to plasma congenitally deficient in factor IX¹⁶. Both factor IX antigen and activity are expressed in arbitrary units; 1 unit corresponds to the quantity of factor IX in 1 ml of normal human pooled plasma ($\sim 5 \mu$ g).

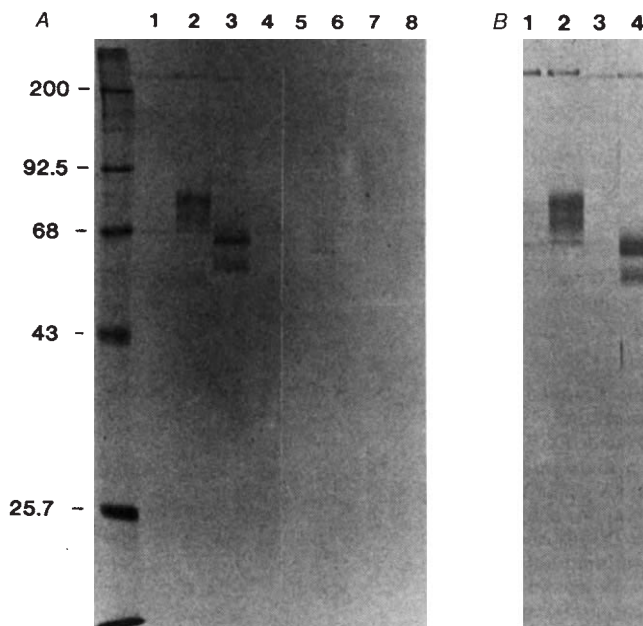


Fig. 3 Immunoprecipitation of recombinant human factor IX. **A**, Immunoprecipitates with labelled cell media. Lane 1, medium from cells infected with wild-type virus; lane 2, medium of cells infected with recombinant virus; lane 3, as for lane 2, but cells grown in tunicamycin; lane 4, as for lane 1, but cells grown in the presence of tunicamycin; lanes 5–8, as for lanes 1–4, but non-immune IgG used instead of anti-factor IX IgG. Numbers on the left represent M_r markers ($\times 10^{-3}$). **B**, Immunoprecipitation with native factor IX. Competitions were performed by adding 10 μ g of natural human factor IX to cell media or extracts before IgG addition. Lane 1, medium from cells precipitated in the presence of competitor; lane 2, as for lane 1, but without competitor; lane 3, medium from tunicamycin-treated cells precipitated in the presence of competitor; lane 4, as for lane 3, but without competitor.

Methods. Confluent hepatoma cells were infected with either recombinant or wild-type vaccinia virus, either in the presence or absence of $1 \mu\text{g ml}^{-1}$ tunicamycin. After 24 h of infection, the medium was removed and replaced by a synthetic medium lacking cold methionine. ^{35}S -methionine (specific activity $\geq 800 \text{ Ci mmol}^{-1}$) was added at $50 \mu\text{Ci ml}^{-1}$, and labelling was carried out for 5 h to obtain labelled supernatants. For cells pretreated with tunicamycin, the compound was also present at $1 \mu\text{g ml}^{-1}$ in the labelling medium. Cells and media were collected, the cells were lysed in 1% Triton X-100 and aliquots of the medium (10^5 c.p.m. of trichloroacetic acid-precipitable radioactivity) were subjected to immunoprecipitation¹⁷ with rabbit anti-human factor IX IgG or non-immune rabbit IgG. The immunoprecipitates were analysed by 7.5% polyacrylamide gel electrophoresis¹⁸, followed by fluorography and autoradiography.

We infected both HepG2 and the mouse fibroblast LM-TK⁻ cell lines with either the wild type or the recombinant vaccinia virus and analysed the medium at several times after adsorption of the virus (Table 1a). Although the factor IX complementary DNA is under the control of the early 7.5K promoter, in the recombinant vaccinia virus construct, we obtained maximal expression of both antigen and activity after ~ 22 h of infection. In fact, the 7.5K promoter is known to be expressed during the entire viral cycle, so the high levels of late expression probably appear after the replication of the viral genome. In medium from cells infected with wild-type vaccinia virus, no significant levels of either factor IX antigen or activity were observed. It is also significant that although there is a good correlation between antigen and activity in the barium citrate-adsorbed fraction for HepG2 cells, this is not the case for the LM-TK⁻ cells (see below). The differences in absolute levels of factor IX produced probably do not result from differences in expression in the two different cell lines, but rather correlate with the

Table 1 Time course of factor IX production (a) and effect of vitamin K (b)

Cell line		HepG2						LM-TK ⁻			
a Virus		w.t.			rec.			rec.		w.t.	
		4	8	22	4	8	22	6	16	22	22
Time after infection (h)		4	8	22	4	8	22	6	16	22	22
Units factor IX non-adsorbed to barium (antigen)		0.01≤	0.01≤	0.01≤	0.03	0.04	0.01	0.09	0.05	0.14	0.01≤
Units of factor IX adsorbed to barium (antigen)		0.01≤	0.01≤	0.01≤	0.01	0.03	0.71	0.02	0.04	0.40	0.01≤
Units of factor IX adsorbed to barium (activity)		0.01	0.01	0.01	0.03	0.04	0.57	0.03	0.04	0.12	0.04
b Virus		w.t.		rec.		w.t.		rec.		w.t.	
Vitamin K added to medium		—	—	—	+	+	—	—	—	+	+
Total factor IX in medium (antigen)		≤0.1	2.1	≤0.1	1.9	ND	ND	ND	ND	ND	ND
Factor IX non-adsorbed to barium (antigen)		0.01	0.43	0.01	0.04	1.06	0.01	0.18	0.01	0.18	0.18
Factor IX adsorbed to barium (antigen)		≤0.01	0.49	≤0.1	0.77	0.16	≤0.01	0.66	≤0.01	0.66	0.66
Factor IX adsorbed to barium (activity)		0.01	0.18	0.02	0.62	0.05	0.02	0.20	0.05	0.02	0.20

a, HepG2 and LM-TK⁻ cells were grown to confluence on 75-cm² plates (~2 and 1 × 10⁷ cells, respectively), then infected with the recombinant factor IX-containing vaccinia virus. These infections were performed in modified Eagle's (MEM) medium supplemented with 10% fetal calf serum (FCS) and 50 µg ml⁻¹ vitamin K. The medium was removed at various times after infection, fractionated using barium citrate as described in the text, and factor IX antigen and activity were measured. b, HepG2 cells were passaged five times in MEM supplemented with 2% serum substitute Ultrosor G (IBF) to lower endogenous vitamin K levels of the cells. Then confluent monolayers in the same medium were infected with the recombinant vaccinia virus, either in the presence or absence of 50 µg ml⁻¹ vitamin K. For the LM-TK⁻ cells, confluent monolayers grown in MEM + 10% FCS were infected in the presence or absence of added vitamin K (50 µg ml⁻¹). Cell media were fractionated, and factor IX antigen and activity were measured as above. For these experiments vitamin K was dissolved in ethanol (50 mg ml⁻¹), then added to the medium. The results are expressed in units, 1 unit corresponding to the quantity of factor IX in 1 ml of normal human pooled plasma. w.t., Wild-type N7 Copenhagen vaccinia virus; rec., recombinant virus; ND, not determined.

number of cells used. Subsequent experiments were performed using medium from cells infected for ~24 h.

As γ-carboxylation of the glutamic acid residues of factor IX is vitamin K-dependent, we investigated the effect of vitamin K on the production of recombinant factor IX (Table 1b). Vitamin K had no effect on the total production of factor IX by HepG2 cells infected with vaccinia, in contrast to the vitamin K-dependent stimulation of prothrombin synthesis reported for this cell line⁶. However, the vitamin did have a marked effect on the distribution of factor IX between the two fractions produced by barium citrate fractionation. When vitamin K was not added, a large part of the synthesized factor IX did not adsorb to barium citrate, but when it was present most of the factor IX bound to the salt.

The factor IX molecules produced by infected HepG2 cells were fully active if vitamin K was present in excess in the medium, but when it was absent the specific activity of factor IX adsorbed to barium citrate was halved. We propose that partially carboxylated proteins can bind to the barium salt, but exhibit a lower biological activity. Similar results were obtained with LM-TK⁻ infected cells, but a striking difference was seen between the factor IX produced by these cells and that from HepG2 cells. Even with an excess of vitamin K in the medium, the molecules produced by infected LM-TK⁻ cells and adsorbed to barium citrate appeared to be three times less active than natural factor IX. As we also found a significant amount of factor IX antigen in the non-adsorbed fraction, we consider that the γ-carboxylation must be less efficient in this cell line. This would explain why the factor produced by LM-TK⁻ cells, even in the presence of vitamin K, was not fully active.

We have demonstrated here the feasibility of producing biologically active factor IX in mammalian cells. The concentration

of factor IX activity in supernatants of infected cells is less than that present in pooled human plasma, but this represents a first step towards the production of a highly purified recombinant protein that could be used to treat haemophilia B.

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Expression of active human factor IX in transfected cells

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Factor IX is the precursor of a serine protease that functions in the intrinsic blood clotting pathway¹. Deficiencies in this plasma glycoprotein result in haemophilia B (or Christmas disease) and occur in about 1 in 30,000 males². Patients are currently treated with fresh frozen plasma or prothrombin complex concentrates prepared from pooled plasma from normal individuals. There are several problems with this method of treatment, including the probable exposure of the patients to contaminants such as the viral agents responsible for hepatitis and AIDS (acquired immune deficiency syndrome). As a first step towards an alternative source of pure human factor IX, we report here on the use of recombinant DNA techniques to produce biologically active factor IX in cultured mammalian cells. Stable cell lines were produced by co-transfecting a baby hamster kidney (BHK) cell line with a plasmid containing a gene for factor IX and a plasmid containing a selectable marker. Protein secreted by these cell lines reduces the clotting time of plasma from factor IX-deficient patients. We present additional evidence that this protein is authentic human factor IX.

We report here the expression of an active vitamin K-dependent protein using recombinant DNA techniques. Factor IX is one of six identified plasma proteins whose biosynthesis is dependent on this vitamin^{1,3}. Vitamin K is a cofactor of the microsomal enzymes that perform post-translational modifications to produce the γ -carboxyglutamic acid (gla) residues characteristic of these proteins. The gla residues, which bind Ca^{2+} and allow the protein to interact with phospholipid, are necessary for biological activity⁴.

Human factor IX circulates in the blood as a single-chain glycoprotein of M_r 57,000 (ref. 1). During blood coagulation, it is converted to an active serine protease (factor IXa) after cleavage by factor XIa. Factor IXa, which consists of a light chain and a heavy chain (M_r 16,000 and 29,000, respectively) held together by a disulphide bond(s), then converts factor X to factor IXa in the presence of factor VIIIa, phospholipid and Ca^{2+} (ref. 1).

To express factor IX in cultured cells, we used a complementary DNA⁵ containing 138 base pairs (bp) that encode the 46-amino-acid leader, 1,248 bp that encode the 415-amino-acid mature protein and 48 bp of 3' noncoding DNA sequence. As described in Fig. 1 legend, this cDNA was inserted into a plasmid containing the adenovirus-2 major late promoter, a set of RNA splice sites and the simian virus 40 (SV40) polyadenylation signal. BHK cells were co-transfected with the factor IX plasmid (FIX/pD2) and pKOneo, a plasmid containing the gene for resistance to the antibiotic G418 (ref. 6). G418-resistant colonies were isolated and screened for the presence of factor IX activity secreted into the media; ~50% of the resistant colonies analysed secreted detectable factor IX activity. In subsequent analyses of a representative clone (IX-F), the supernatant media and the cell pellets of clone IX-F were assayed for the amounts of biologically active factor IX and factor IX polypeptide. The assay for biological activity was based on the ability of the sample to reduce the blood clotting time of plasma from factor IX-deficient patients⁷ and the amount of factor IX polypeptide was determined by an enzyme-linked immunosorbent assay (ELISA) using an affinity-purified polyclonal rabbit antibody to factor IX. The cells were grown for different lengths of time, and the media and cells collected for analysis. Most of the factor

Table 1 Production of factor IX by cell line IX-F

Day	Cell no. $\times 10^4$ per ml of medium	Factor IX activity in medium (ng ml ⁻¹)	Factor IX polypeptide (ng ml ⁻¹) Supernatant Cell pellet	% Active protein in medium
1	1.86 1.44	ND	ND	ND
2	2.73 2.58	27 24	57 45	20 20
3	9.99 9.39	72 84	150 120	60 60
4	17.58 12.00	198 150	475 225	160 140
5	48.90 52.80	408 438	875 1,000	250 260
6	66.00 72.00	648 678	1,500 1,375	220 290

Cells from clone IX-F were plated at 1.5×10^5 cells per 10-cm dish in Dulbecco's modified Eagle's medium containing 10% fetal calf serum and $1 \mu\text{g ml}^{-1}$ vitamin K (phytonadione, Merck). At the indicated time, the medium was aspirated, centrifuged at 2,000 r.p.m. for 10 min and frozen at -80°C . The cells were trypsinized, washed twice with Dulbecco's medium and counted. The cell pellet was frozen at -80°C . For the protein assays, the supernatant samples were thawed and assayed for biological activity by a one-stage clotting assay⁷. This assay is based on the ability of the sample to decrease the prolonged activated partial thromboplastin time of congenital factor IX-deficient plasma. Normal control plasma (0.1 ml) (George King Biomedical Co.) or supernatant medium (0.1 ml) was incubated with factor IX-deficient plasma (0.1 ml) (George King Biomedical Co.) and cephaline-kaolin mixture⁷ (0.1 ml) for 12 min at 37°C . Then 40 mM CaCl_2 (0.1 ml) was added and the clotting time recorded. The factor IX activity was expressed as a percentage of the activity present in normal plasma and then converted to ng ml^{-1} using a standard value of $3 \mu\text{g ml}^{-1}$ of factor IX in normal plasma. The cell pellets were extracted by freeze-thawing three times in 2 ml phosphate-buffered saline (PBS) and then centrifuged for 5 min in a microfuge. The pellet was re-extracted in 1 ml PBS, 0.25% Triton X-100. The samples were diluted 1:5 for ELISA. The amount of factor IX polypeptide present in the samples was measured by ELISA as follows. Affinity-purified rabbit polyclonal antibody against human factor IX ($200 \mu\text{l}$ of $1 \mu\text{g ml}^{-1}$ in 0.1 M Na_2CO_3 , pH 9.6) was incubated in each well of 96-well microtitre plates overnight at 4°C or for 2 h at 37°C . The wells were washed three times with PBS, 0.05% Tween 20 and then incubated with $220 \mu\text{l}$ of 1% bovine serum albumin (BSA), 0.05% Tween 20 in PBS, pH 7.2 overnight at 4°C or for 2 h at 37°C . The plates were rinsed several times in distilled water, air-dried and stored at 4°C . To assay samples containing factor IX, $200 \mu\text{l}$ of each sample was incubated for 1 h at room temperature in the antibody-coated wells. The wells were rinsed four times with 0.05% Tween 20 in PBS and then incubated for 1 h at room temperature with affinity-purified rabbit polyclonal anti-factor IX conjugated to alkaline phosphatase in PBS containing 1% BSA and 0.05% Tween 20. The per cent active protein was calculated by dividing the amount of factor IX activity in the medium by the amount of factor IX polypeptide in the medium. The same analyses were performed on spent media and cell pellets from control BHK cells and no factor IX was detected. To produce clone IX-F, the plasmid FIX/pD2 was introduced into BHK cells by calcium phosphate-mediated transfection¹¹. To transfect one 100-mm dish of 20% confluent cells, a total of $20 \mu\text{g}$ DNA was used: $5 \mu\text{g}$ of FIX/pD2, $5 \mu\text{g}$ pKOneo and $10 \mu\text{g}$ salmon sperm DNA. Twenty-four hours after transfection, selective medium (0.5 mg ml^{-1} , Gibco G418) was added. Resistant colonies were picked after 10–13 days. ND, not detectable.

IX polypeptide (70–80%) was secreted into the media, and ~50% of the secreted protein was biologically active (Table 1). No biological activity was detected in the cellular extracts despite the presence of factor IX polypeptide. Extracts of BHK cells had no inhibitory effect on the clotting assay.

It was necessary to supplement the cell culture medium with vitamin K at $1\text{--}10 \mu\text{g ml}^{-1}$ to detect factor IX clotting activity (data not shown). In the absence of vitamin K, the cells secreted factor IX polypeptide at somewhat higher levels than when vitamin K was present. However, without vitamin K, little or no factor IX clotting activity was detected.

Additional analyses were performed to demonstrate that these cells were secreting factor IX. Samples containing factor IX activity as measured by the above assay were incubated with factor VIII-deficient plasma; none of these samples shortened the clotting times of factor VIII-deficient plasma (data not shown). Therefore, it seemed that the activity was due to factor IX rather than an artefactual agent. This conclusion was confirmed by the finding that treatment of the samples with an affinity-purified rabbit polyclonal antibody against human

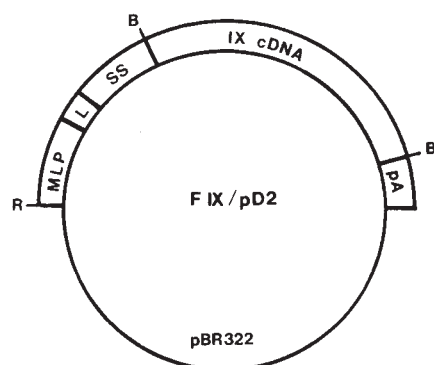


Fig. 1 Diagram of the factor IX construction used in our experiments. The factor IX cDNA obtained by Kurachi and Davie⁵ begins with the first methionine (ATG) of the protein leader sequence¹². The sequence of the 5' end was altered from CTGCAGG₁₁ ATG to CTGCAGGATCCATG to remove the G₁₁ and to introduce a *Bam*HI site 5' of the ATG. An adaptor was made by annealing two synthetic single-stranded DNAs, filling-in with T4 polymerase, and then digesting with the restriction enzymes *Pst*I and *Cfo*I. The resulting 19-bp *Pst*I/*Cfo*I fragment was ligated with a 1.4-kb *Cfo*I/*Bam*HI fragment which contained the remainder of the factor IX cDNA and a 2.7-kb *Bam*HI/*Pst*I fragment of pUC13. The DNA construction was confirmed by sequencing through the region where the adaptor was inserted. The cDNA was then isolated as a *Bam*HI fragment and inserted into the *Bam*HI site in the vector pD2 to make FIX/pD2. The pD2 plasmid (5.3 kb) was derived from plasmid pDHFR-III (ref. 13) and contains the adenovirus-2 major late promoter (MLP), the cDNA encoding the entire tripartite leader (L), a splice set (SS) composed of the adenovirus-2 third leader 5' splice site and an immunoglobulin 3' splice site, and the early SV40 polyadenylation signal (pA). R, *Eco*RI restriction enzyme site; B, *Bam*HI site.

factor IX removed 98% of the factor IX activity. This antibody also precipitated 99% of the factor IX activity from normal plasma. When the samples were immunoprecipitated with a rabbit polyclonal antibody to human erythropoietin, none of the factor IX activity was removed from the samples.

Barium citrate has been used to adsorb selectively proteins containing gla residues⁸. Culture medium from cell line IX-F was subjected to adsorption as described in Table 2 legend. The supernatant and the pellet fractions were assayed by the clotting assay and by ELISA. The ELISA data (Table 2) show that while 50% of the factor IX polypeptide was adsorbed to barium citrate, almost all of the biologically active factor IX was adsorbed. These data suggest that the biologically active factor IX was properly carboxylated (and therefore adsorbed to barium citrate), whereas the inactive factor IX was probably deficient in gla residues. Human factor IX isolated from plasma contains 12 gla residues⁹. Protein sequence and amino-acid composition analyses of our active and inactive factor IX fraction will be necessary to determine precisely the extent of carboxylation in the factor IX produced by BHK cells.

A Western blot analysis was performed on the material adsorbed to barium citrate from spent media of cell line IX-F and BHK cells, and from normal plasma. The protein secreted by IX-F (Fig. 2, lane 2) migrated with the same apparent M_r as the single-chain form of factor IX in normal plasma (lane 3) and of purified factor IX (lane 4). These results indicate that the cells secreted human factor IX of the correct size and that the single-chain form was stable in the cell culture medium for up to 6 days.

The present work demonstrates that biologically active human factor IX can be produced in BHK cells. Additional studies are in progress to increase the levels of protein secretion and to optimize the expression of active factor IX in transfected cells. Since variation has been observed in the rate of carboxylation by microsomal fractions from different tissues¹⁰, one parameter

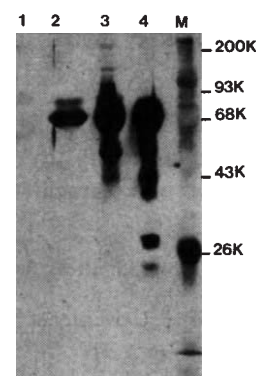


Fig. 2 Western blot of factor IX adsorbed to barium citrate. Spent media were collected from BHK cells and from IX-F cells grown for 6 days as described previously. Aliquots (2 ml) of each medium sample and normal plasma (2 ml) were adjusted to 23.8 mM sodium citrate and incubated at 4 °C for 5 min. Barium chloride was added to a final concentration of 45.5 mM, incubated at 4 °C for 30 min and centrifuged at 3,000 r.p.m. for 2 min. The pellets were washed twice with PBS and redissolved in 2 ml of PBS containing 0.15 M sodium citrate. The IX-F sample contained 400 ng ml⁻¹ factor IX by ELISA, and the control BHK samples contained none. The samples were dialysed for 4 h at 4 °C against PBS and then concentrated using Aquacide III (Calbiochem-Behring) to a volume of 300 μ l. The control BHK sample (lane 1), the IX-F sample (75 μ l; 75 ng of factor IX) (lane 2), normal plasma (20 μ l; 400 ng of factor IX) (lane 3) and PBS (50 μ l) containing 1 μ g of purified factor IX (lane 4) were subjected to electrophoresis in a 10% polyacrylamide gel (acryl:bis, 3.75:1). After the proteins had been transferred to nitrocellulose, the filter was washed with transfer buffer A (50 mM Tris-HCl pH 7.4, 5 mM EDTA, 50 mM NaCl, 0.25% (w/w) gelatin, 0.05% NP40) and incubated with rabbit polyclonal antibody to factor IX for 1 h at room temperature. After three more 15-min washes with buffer A, the filter was incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase (HRP) for 1 h at room temperature, washed once with distilled H₂O, twice with buffer B (same as buffer A but also containing 0.4% N-lauryl sarcosine sodium salt) for 15 min, and finally incubated with HRP colour reagent (Bio-Rad). The factor IX in all lanes migrated with an apparent M_r of 66,000. Presumably this difference from the reported 57,000 M_r results from the electrophoretic properties of factor IX in this system. Some degradation of the purified factor IX standard (lane 4) was apparent. The M_r standards (BRL, pre-stained with Coomassie blue) (lane M) are myosin heavy chain (200,000 or 200K), phosphorylase b (92,500), BSA (68,000), ovalbumin (43,000) and α -chymotrypsin (25,700).

Table 2 Barium citrate adsorption of factor IX from cell culture medium

Sample	Factor IX (ng) (ELISA)	Factor IX (ng) (activity)
Medium from clone IX-F	5,288	3,186
Supernatant fraction after precipitation and dialysis	2,641	14
Redissolved precipitate	1,500	1,935

Spent media were collected from cell line IX-F and assayed by ELISA and by the clotting assay. A 4.8-ml aliquot of medium was adjusted to 20 mM sodium citrate and the sample was incubated for 5 min at 4 °C. Barium chloride was added to a final concentration of 40 mM and the sample was incubated at 4 °C for 30 min and then centrifuged at 3,000 r.p.m. for 2 min. The supernatant was dialysed against PBS at 4 °C, and then assayed. The pellet of insoluble barium citrate was redissolved in PBS containing 0.1% BSA and 0.15 M sodium citrate, dialysed against PBS and then assayed. According to both the activity assay and the ELISA, the per cent recovery of protein from the barium citrate pellet in this experiment was about 60%. We found that the per cent recovery varied between experiments, but the activity and the ELISA measurements were always consistent. Spent media collected from control BHK cells were subjected to the same analysis, and no factor IX activity or ELISA-positive material was detected.

to explore is the efficiency of γ -carboxylation in other cultured cell lines. From these initial studies, however, it is clear that this approach provides the basis for establishing an abundant pure source of human factor IX.

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Isolation of a new human oncogene from a diffuse B-cell lymphoma

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Utilizing DNA transfection analysis¹ with the continuous NIH 3T3 cell line as assay cell², we³ and others^{4,5} have observed that as many as 10-50% of human haematopoietic tumours contain oncogenes, the vast majority of which are members of the *ras* proto-oncogene family. In addition, Cooper and co-workers⁶⁻⁸ have reported the detection and isolation of specific oncogenes, *B-lym* and *T-lym*, which appear to be activated in human and rodent tumours of certain B and T lymphoid cells, respectively. In surveying human haematopoietic malignancies, we observed that DNA of a primary human diffuse B-cell lymphoma induced an unusual transformed focus on transfection of NIH 3T3 cells. Here, we report the molecular cloning and physical characterization of this human oncogene, whose transforming activity was shown to reside within a human DNA sequence of 45 kilobases (kb) cloned in a cosmid vector. Its properties distinguish it from previously reported retroviral or nonretroviral oncogenes.

High relative molecular mass (M_r) DNA was isolated from the affected spleen of a patient with diffuse B-cell lymphoma. When the DNA was tested in the NIH 3T3 transfection assay, transforming activity was observed at low efficiency. The transformed cells demonstrated a very distinct phenotype (Fig. 1). At least three types of morphologically distinguishable cells were visible: giant polynucleated cells, densely growing fibroblast-like cells and very refractile and round cells (Fig. 1). The unusual phenotype of the transformed focus appeared to be a genetically stable property. Clonally derived lines retained the complex morphology of the originally observed transformed focus.

A first-cycle focus was picked and the transformant grown up to mass culture. DNA of the primary transfectant was shown to contain human repetitive sequences, indicating that the transfectant had incorporated exogenous human DNA. Transmissibility of the transformed phenotype was retained in subsequent cycles of transfection with concomitant increased efficiency (Table 1). Moreover, cell lines established from first-, second- and third-cycle transfectants induced by this human transform-

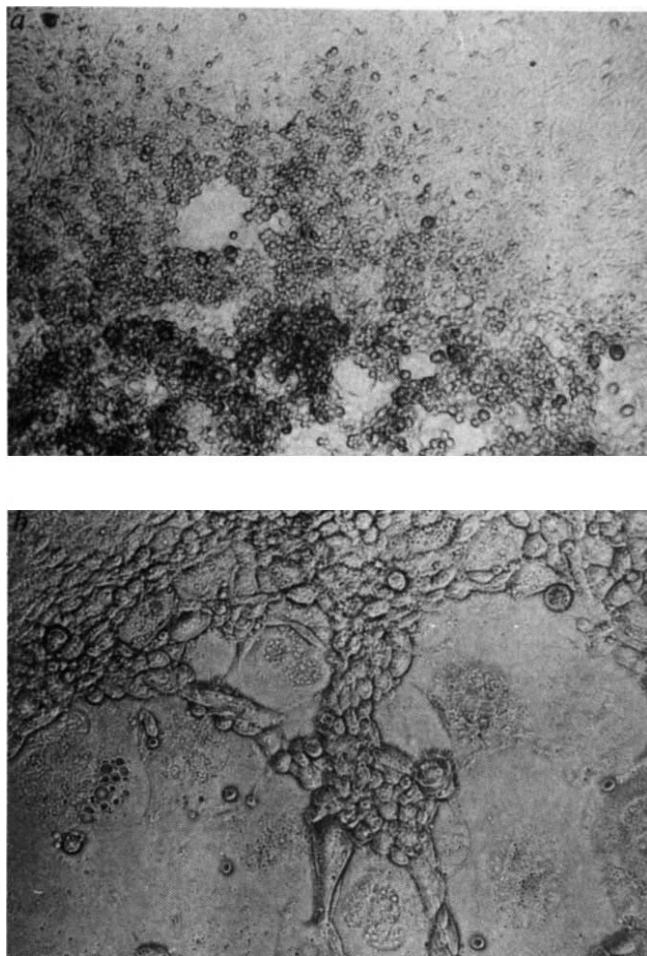


Fig. 1 Morphology of a transformed focus induced in NIH 3T3 cells by transfection of high- M_r DNA from a human diffuse B-cell lymphoma. a, $\times 140$; b, $\times 360$.

ing gene possessed known properties of malignant cells (Table 1).

We then analysed second- and third-cycle transfectants by Southern blotting analysis and hybridization with a probe specific for human repetitive sequences. As shown in Fig. 2, human sequences were detected in each transfectant analysed. The pattern of human DNA fragments observed following digestion with different enzymes indicated the retention of specific human fragments through several cycles of transfection, implying their probable association with the transforming gene.

Most human transforming genes detected by transfection analysis to date have been found to be members of the *ras* family, with *N-ras* the most frequently activated oncogene in human haematopoietic tumours³⁻⁵. To exclude the possibility that the newly detected oncogene was related to any of the known *ras* genes, we analysed transfectant DNAs by Southern blotting for the presence of human *ras*-related sequences. None of the *ras* gene probes detected related sequences other than those normally present in mouse cells (data not shown).

A third-cycle transfectant DNA was partially digested with *EcoRI*, selected by sucrose gradient for the size range of 35-45 kb and cloned into cosmid pH79. A library of 300,000 recombinants was screened with human genomic DNA as a probe. Four overlapping clones were isolated (C14-1-2, C5-1-1, C44-1-6, and C8X-1-1, Fig. 3a), whose 40-45-kb inserts contained human repetitive sequences. A preliminary restriction map was constructed and is shown in Fig. 3b. *EcoRI* and *HindIII*, which cut each of the inserts a minimum of nine times, were partially mapped, so only those *EcoRI* and *HindIII* sites that were

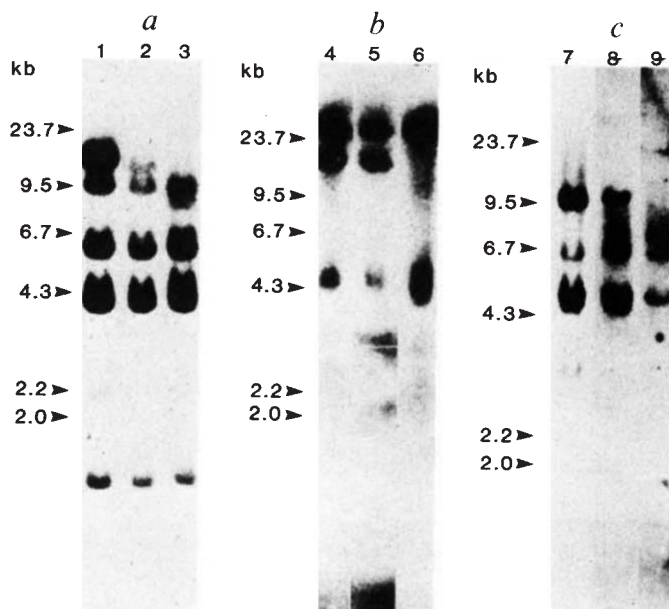


Fig. 2 Detection of human sequences in NIH 3T3 cells transformed by DNA of a diffuse B-cell lymphoma. High M_r DNA (20 μ g) was digested with *Eco*RI (a), *Bam*HI (b) or *Hind*III (c), electrophoresed, blotted and hybridized to 32 P-labelled nick-translated 0.3-kb cloned repetitive sequence DNA fragment purified from plasmid BLUR-8 (2×10^6 c.p.m. ml^{-1}). Cellular DNA was isolated from one representative second-cycle transfectant (lanes 1, 4, 7) and two representative third-cycle transfectants (lanes 2, 5, 8 and 3, 6, 9, respectively). *Hind*III fragments of λ DNA served as M_r standards.

determined are indicated. As shown in Fig. 3c, two of the clones, C5-1-1 and C14-1-2, contained only human sequences and were placed in the map arbitrarily at the left end of the cloned DNA sequences. The other two clones, C44-1-6 and C8X-1-1, contained both human and mouse repetitive sequences and thus probably represented one of the junctions of the human acquired sequence with mouse DNA.

To establish whether any of the clones possessed transforming activity, we transfected NIH 3T3 cells with varying amounts of each of the recombinant cosmid DNAs. C14-1-2, which contained a 45-kb human DNA fragment, was found to be biologically active in three separate experiments using two different DNA preparations with an efficiency of focus formation of about 600 focus forming units per pmol (data not shown). The morphology of transformed foci induced by the cloned oncogene was indistinguishable from that observed by transfecting NIH 3T3 with the original human tumour or transfectant DNAs.

DNAs of representative foci obtained by transfection with C14-1-2 DNA were analysed by Southern blotting using as probes a 4.5-kb *Bam*HI fragment that maps at the right end of C14-1-2 (Fig. 3a). We found that this probe recognized DNA fragments of the sizes expected from the physical map of C14-1-2 in all the transfectant DNAs analysed. All of these findings established that the C14-1-2 recombinant cosmid clone contained the biologically active transforming gene.

We hybridized third-cycle transfectant DNAs with cloned viral and cellular oncogenes and performed reciprocal hybridization studies between these probes and the cloned oncogene. In addition to its lack of homology with any of the known *ras* genes, the transforming gene lacked detectable relationship to *sis*, *rel*, *fos*, *erb-B*, *B-lym*, *N-myc*, *fgr*, *fes*, *src*, *ets*, *fms*, *p53*, *myb*, *mos*, *abl*, *myc* and *raf* (data not shown). Furthermore, its restriction map was different from that of T-lym⁸ as well as that recently reported for *met*⁹. Finally, comparison of the pattern of *Alu*-containing fragments of second- and third-cycle transfectants with the human specific fragments of *met*⁹, *mcf-2*¹⁰, *mcf-3*¹⁰,

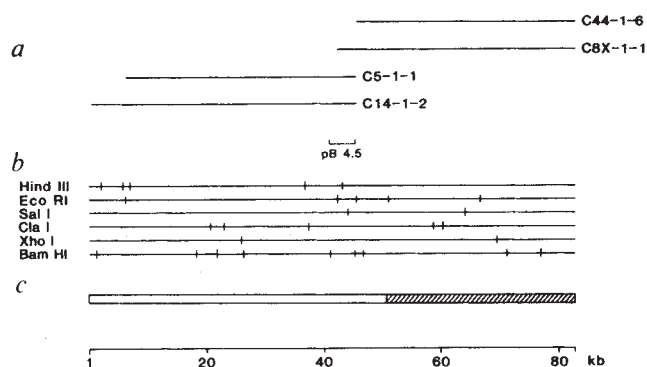


Fig. 3 Restriction map of *dbl*. a, Genomic DNA of a third-cycle transfectant was partially digested with *Eco*RI to select for fragments of from 35–45 kb and cloned in pHC79 cosmid cloning vector. The library was screened without amplification with 32 P-labelled nick-translated, sheared human genomic DNA as a probe. The four overlapping recombinant clones obtained are indicated. A *Bam*HI restriction fragment of cosmid clone C14-1-2 was subcloned in pAT153 (subclone pB 4.5). b, Restriction map of the cloned cellular DNA. *Hind*III and *Eco*RI were only partially mapped. c, Human (open box) and mouse (hatched box) specific sequences were determined by hybridization with 32 P-labelled nick-translated, human genomic DNA, human *Alu* clone BLUR-8 and mouse genomic DNA.

NK14¹¹ and *neu* oncogenes¹² indicated a lack of homology between our transforming gene and any of these recently detected oncogenes.

Our present studies provide the first report of a transforming gene associated with a human diffuse B-cell lymphoma. This newly identified oncogene, which we designate *dbl* for diffuse B-cell lymphoma, conferred the transformed phenotype on NIH 3T3 cells, inducing an unusual focus composed of small and spindle-shaped refractile cells as well as large multinucleated giant cells. Despite the low transforming activity of the original human tumour DNA, we presume that the transforming gene arose from within the tumour, as we have not observed transformed foci induced by normal human DNA in a large series of transfection experiments. However, direct confirmation must await further characterization of the structure of the oncogene as it resides within the tumour. The distinct phenotype

Table 1 Transforming properties of DNA from a human diffuse B-cell lymphoma

Transforming activity	Properties of transformed cells			
	No. foci per plate*	Saturation density (cells $\times 10^{-5}$ per cm^2)†	% Colony formation in agar‡	Tumour incidence§
Donor DNA				
I cycle	0.12	2.0–3.0	5–10	5/5
II cycle	1.5	2.0–3.0	5–10	5/5
III cycle	3.0	2.0–3.0	5–10	5/5
Control				
NIH 3T3	<0.03	1.0	<0.01	0/5

* 1.5×10^5 NIH 3T3 were transfected with 40 μ g of high M_r DNA by the calcium phosphate method¹. Results represent mean values of at least eight recipient cultures.

† The maximum cell number obtained when 5×10^3 cells per cm^2 were inoculated onto 20- cm^2 Petri dishes in conditions where the medium containing 5% calf serum was changed every 3 days. Saturation density was taken as that value where successive cell counts at 2-day intervals showed no increase in cell number.

‡ Cell suspensions were plated at 10-fold serial dilutions in 0.33% soft agar medium containing 10% calf serum. Visible colonies comprising >100 cells were scored at 14 days.

§ NFR nude mice were inoculated subcutaneously with 1×10^5 cells of each cell line. Tumour formation was monitored for up to 30 days.

of transformants induced by this oncogene suggests that the NIH 3T3 transfection assay may reveal other oncogenes which induce alterations that differ from those caused by *ras* oncogenes.

Our isolation of a 45-kb cosmid clone comprised of human sequences, which induced transformation on DNA transfection, demonstrates that the transforming gene was obtained in a biologically intact form. This clone was only one of four isolated by cosmid cloning of *Eco*RI partially digested genomic DNA. Thus, it is likely that restriction sites outside the transforming region were preferentially cleaved by the cloning enzyme. To our knowledge this 45-kb DNA sequence represents the largest biologically active transforming DNA so far isolated.

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Control of transcription of α -amylase and rRNA genes in barley aleurone protoplasts by gibberellin and abscisic acid

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Gibberellin A₃ (GA₃) promotes and abscisic acid (ABA) inhibits the production of α -amylase in aleurone layers of barley (*Hordeum vulgare* L.); this system has been used extensively to study the mechanisms of action of these hormones¹. Cell-free messenger RNA translation studies have shown that the GA₃-promoted increase in α -amylase synthesis and its inhibition by ABA are associated with corresponding changes in the levels of translatable α -amylase mRNA²⁻⁵. Subsequent studies using hybridization of an α -amylase complementary DNA-rich probe⁶ and of cloned α -amylase cDNA probes to aleurone RNA⁷⁻⁹ have shown that the abundance of α -amylase mRNA increases in response to GA₃, and that this increase is inhibited by ABA. The increased abundance of mRNA could result from an increased rate of transcription, increased stability of mRNA, or both, as in some animal cells¹⁰⁻¹². By using methods for studying gene transcription in isolated plant nuclei¹³⁻¹⁵, we present here evidence that GA₃ and ABA regulate the transcription of both α -amylase and ribosomal RNA (rRNA) genes.

Nuclei were isolated from GA₃-responsive aleurone cell protoplasts¹⁶ and to facilitate this, several minor changes were made to our earlier protoplast preparation procedure. Figure 1 shows that, using the modified procedure, very little α -amylase activity accumulated in control protoplasts, that there was a marked

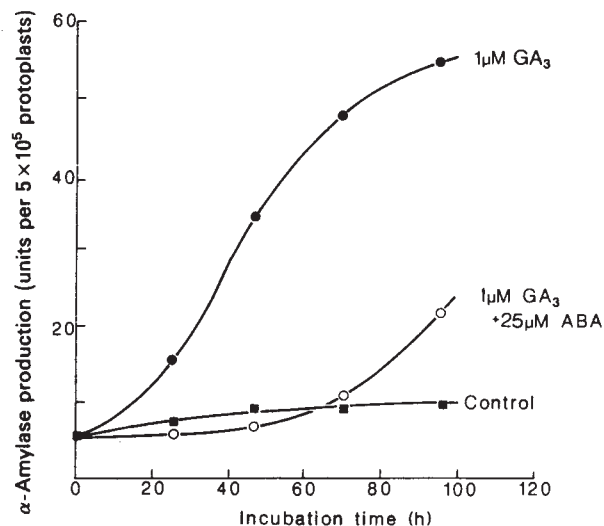


Fig. 1 Regulation of α -amylase produced by barley aleurone protoplasts in response to GA₃ and ABA.

Methods. Protoplasts were prepared from aleurone layers of barley (*H. vulgare* cv. Himalaya) under nitrogen as in an earlier study¹⁶ but 20 quarter grains, not 10, were placed in each Erlenmeyer flask and the volume of protoplast isolation medium containing cellulase Onozuka R-10 was increased from 1 ml to 1.5 ml; this procedure gave about 500,000 protoplasts per flask. Flasks received 100 μ l of either water (control, $\blacksquare$) or a solution of GA₃ (final concentration 1 μ M, $\bullet$) or GA₃ plus *cis*, *trans*-ABA (final concentration 25 μ M) ($\circ$) and were left without shaking at room temperature ($22 \pm 2^\circ\text{C}$). Assay and expression of α -amylase activity have been described elsewhere¹⁶. Values represent the mean of four flasks of protoplasts.

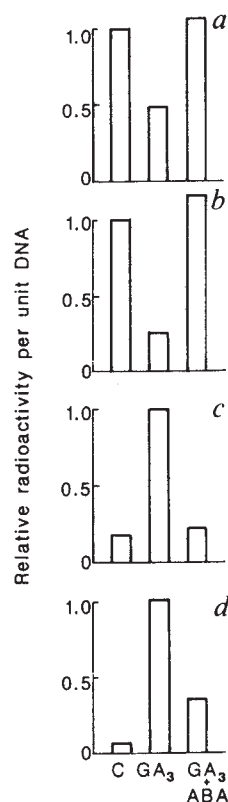


Fig. 2 Comparison of the effects of hormones on accumulation of total (a) and various specific transcripts (b-d) in isolated nuclei. b, rRNA; c, α -amylase; d, RNA specified by pHV14. The relative values for each RNA species were calculated from the values of c.p.m. per 100 μ g DNA given in Table 1. c, control nuclei; GA₃, nuclei from GA₃-treated protoplasts; GA₃ + ABA, nuclei from protoplasts treated with both GA₃ and ABA.

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Table 1 Accumulation of transcripts by nuclei isolated from barley aleurone protoplasts incubated for 24 h with and without GA₃ and ABA

	Control	GA ₃	GA ₃ + ABA
Total transcription	7,530 (100)	3,712 (100)	8,075 (100)
rRNA	1,355 (18)	360 (9.7)	1,574 (19.5)
α -Amylase transcripts	0.25 (0.0033)	1.37 (0.037)	0.32 (0.0040)
pHV14 transcripts	0.17 (0.0023)	2.52 (0.068)	0.89 (0.011)

The values are (c.p.m. per 100 μ g DNA) $\times 10^{-3}$ and the numbers in parentheses are percentages of total transcription. Protoplasts were prepared and incubated for 24 h with and without hormones as described for Fig. 1 (10 flasks per treatment). Because starch grains interfered with the protoplast lysis procedure (see below), protoplasts were purified on discontinuous Percoll gradients made essentially as described by Luthe and Quatrano¹⁹. The gradients consisted of 5-ml layers of 2 M sucrose (bottom), and of 80% and 15% Percoll in 30-ml Corex centrifuge tubes. The Percoll was made up in 0.44 M sucrose (RNase-free; Bio-Rad), 25 mM Tris-HCl pH 7.5 and 10 mM MgCl₂. The contents of five incubation flasks were layered onto each gradient (on ice) and the tubes were centrifuged at 4,080g (5,000 r.p.m. in a Sorvall HB4 swinging bucket rotor) for 20 min. The protoplasts band at the top and bottom of the 15% Percoll layer, the starch falls to the bottom of the tube, and the dead protoplasts float to the top of the original protoplast medium. The entire 15% Percoll layer was recovered and diluted with an equal volume of 0.44 M sucrose, 25 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 5% w/v Ficoll and 10% w/v Dextran 40. To this mixture was added 1 μ l ml⁻¹ β -mercaptoethanol, 0.5% (v/v) Triton X-100 and 2 mM spermine; the cells were then contained in a solution similar to the modified Honda medium of Luthe and Quatrano¹⁹. The protoplasts were broken by passing the suspension five times through nylon mesh with nominally 20- μ m pores (actually 13 μ m when measured). The nuclei were recovered from solution by centrifugation (3,000 r.p.m. for 10 min in an HB4 rotor) and washed twice in 10 ml of 2.5% Ficoll 400, 5% Dextran T40, 0.25 M sucrose, 0.025 M Tris-HCl pH 7.8, 0.01 M MgCl₂ and 1 μ l ml⁻¹ β -mercaptoethanol. Pellets were resuspended gently using a glass rod and centrifuged at 3,000 r.p.m. in an HB4 rotor for 5 min. The washed pellet derived from 10 flasks of protoplasts was resuspended in ~150 μ l (total volume) of the washing buffer. Transcription reactions were started by adding 120 μ l of nuclei (usually containing ~80 μ g DNA) to 600 μ l of transcription mixture. Final concentrations of components were: 50 mM (NH₄)₂SO₄, 0.45 mM each of ATP, GTP and GTP, and 2 mM MnCl₂. Each reaction contained 300 μ Ci [α -³²P]UTP (specific activity ~3,000 Ci mmol⁻¹; Amersham). Attempts to use [α -³²P]GTP to radiolabel RNA were unsuccessful because transcription was progressively more inhibited as the amount of radioactive GTP in the reaction was increased. The reactions were incubated at 20 °C for 1 h. To measure accumulation of total RNA, aliquots of the reaction mixture were taken at the beginning and end of the reaction and the nucleic acid was precipitated with cold trichloroacetic acid (TCA) in the presence of carrier herring sperm DNA and the radioactivity in RNA determined as described previously¹⁷. To measure specific gene transcripts, the RNA was first extracted from the nuclei. The nuclei, containing 94% of the TCA-precipitable radioactivity, were recovered from the reaction mixture by centrifugation and the RNA was purified essentially as described elsewhere¹⁷, except that the proteinase K and sonication steps were omitted. The ethanol-precipitated nucleic acid was centrifuged and dissolved in 50 μ l of 0.1% SDS, 0.5 mM EDTA and 1 mM Tris-HCl pH 7.5. Recombinant plasmids used to detect specific transcripts were pTA71, containing wheat genomic ribosomal DNA²⁰; pHV19, containing sequences for barley aleurone α -amylase⁹; and pHV14, which is unidentified but contains sequences for a barley polyadenylated RNA species whose concentration increases in the presence of GA₃, as does α -amylase mRNA⁹. The control plasmid was pP15-71, which contains *Pisum sativum* cDNA sequences specific for vicilin²¹. Plasmid DNA (2 μ g) was bound to nitrocellulose filter disks (6.5 mm diameter) by the method of Thomas²². Hybridizations were performed as described previously¹⁷, except that rRNA hybridizations were done in 30 μ l and the others in 100 μ l. All hybridizations contained a pP15-71 filter for the determination of background radioactivity, which was subtracted from that binding to other filters in the hybridization. Hormone treatments did not affect the recovery of DNA from protoplasts but, because they did affect total transcription, hybridized counts have been expressed both as a percentage of total transcriptional activity and as c.p.m. per 100 μ g DNA ($\times 10^{-3}$), as described previously¹⁷.

response to 1 μ M GA₃ and that a 25-fold excess of ABA over GA₃ strongly inhibited α -amylase accumulation, at least up to 48 h, at which time accumulation of the enzyme appeared to be released from ABA control. From these results, we chose to isolate nuclei for *in vitro* transcription experiments from protoplasts incubated with or without hormones for 24 h, as at this time, ABA is strongly inhibitory and the rate of α -amylase accumulation is still increasing. Previous studies indicated that an increasing rate of α -amylase accumulation is associated with increasing levels of α -amylase mRNA^{2,5}. If the increasing levels of α -amylase mRNA are the result of increased transcription of the α -amylase genes, then 24 h should be a suitable time to detect it.

Initially, nuclei were isolated from protoplasts which had been incubated for 24 h with and without GA₃ and ABA (as in Fig. 1) and their capacity for transcription *in vitro* was determined. All nuclei preparations incorporated nucleoside triphosphates into RNA for 1 h, after which acid-precipitable RNA was slowly lost, presumably as a result of ribonuclease activity (data not shown). Accordingly, all transcriptions were run for 1 h, at which time total incorporation of [α -³²P]UTP into RNA reached maximal levels of up to 80,000 c.p.m. per μ g DNA, depending on the treatment (see below).

To determine the effects of hormones on the accumulation of total transcripts and on the accumulation of specific gene transcripts in isolated nuclei, protoplasts were incubated with or without hormones for 24 h, and their nuclei were isolated and allowed to transcribe *in vitro*. The *in vitro*-synthesized RNA was hybridized to specific cloned DNAs (see Table 1). The results in Table 1 are from one experiment but they are representative

of results obtained in two other experiments. Incorporation of [α -³²P]UTP into total RNA was decreased in nuclei isolated from protoplasts treated with GA₃ compared with the level in nuclei from control protoplasts. This decreased incorporation did not occur when protoplasts were treated with ABA in addition to GA₃ (Table 1). Because the concentration of UTP added to the transcription reaction was very low (0.12 μ M), these results could be explained by hormone-mediated effects on the size of the residual UTP pool in isolated nuclei. However, experiments using UTP concentrations 77 times greater showed the same effects of the hormones on total RNA accumulation. Thus, the hormonal effects are not artefacts resulting from changes in specific radioactivity of the *in vitro*-synthesized RNA.

In the above experiment, the rRNA transcripts contributed 18% of the total transcripts in nuclei from untreated protoplasts although in other experiments their contribution was as low as ~6%. The range of these values is similar to that reported for pea leaf nuclei¹³ but considerably less than the range for pea cotyledon nuclei, which was ~45 to 77% (ref. 17). The accumulation of rRNA transcripts in this and other experiments was depressed by about 50% in protoplasts treated with GA₃ but this did not occur if ABA was also present.

The hormones also had large effects on the accumulation of α -amylase gene transcripts. In accordance with the very low level of α -amylase activity and α -amylase mRNA in control protoplasts¹⁶, there was very little accumulation of α -amylase gene transcripts in control nuclei; when GA₃ was present, however, accumulation of the transcripts increased about 11-fold to a level of 0.037% of total transcription. Incubation of proto-

plasts with ABA in addition to GA₃ once again inhibited the effect of GA₃. The α -amylase cDNA clone pHV19 probably corresponds to mRNA for the B group of α -amylase isozymes⁹. We believe that in the hybridization conditions of the present experiments, transcripts of both A and B α -amylase families will hybridize to pHV19 because of the relatively high nucleotide sequence homology between group A and B cDNA clones and because of their high G+C content⁹. Therefore, the α -amylase gene transcripts measured here probably represent the composite contribution of several α -amylase genes, estimated to be at least eight in the *H. vulgare* cv. Himalaya genome^{7,9}. Our results do not allow us to determine whether or not GA₃ regulates the transcripts of all genes equally.

We also measured the accumulation of transcripts specific to clone pHV14 because it specifies a mRNA whose abundance also increases in the presence of GA₃, as does α -amylase mRNA, although we have not identified any protein that it encodes⁹. The effects of GA₃ and ABA on the synthesis of RNA hybridizing to pHV14 were similar to their effects on α -amylase gene transcription (Table 1).

These results leave little doubt that GA₃ and ABA regulate nuclear activity in barley aleurone cells. The most likely explanation for the results is that the hormones regulate specific gene transcription and thus mRNA formation, although it is still possible that altered transcript stability and processing within the nucleus may be involved. The accumulation of α -amylase gene transcripts was increased about six times in nuclei from GA₃-treated protoplasts although in other experiments it was increased by up to 14 times. The variation in the extent of the increase of α -amylase gene transcripts with GA₃ treatment probably reflects the small and variable numbers of hybridizable counts in RNA accumulated by control nuclei. It is of interest to examine whether the hormone-promoted increase of α -amylase transcripts could account for the observed changes in level of α -amylase caused by GA₃. The rate of α -amylase accumulation after 24 h was increased from ~0.053 units h⁻¹ to ~0.63 units h⁻¹, an increase of a factor of ~12 (Fig. 1). Although these values are approximate, the increased concentration of α -amylase gene transcripts is clearly in accord with the increased rate of α -amylase accumulation. However, more detailed studies of rates of gene transcription, mRNA levels, mRNA stability and rates of enzyme synthesis are necessary to determine whether GA₃ controls α -amylase synthesis solely by regulating gene transcription.

A review of the literature shows that ABA has been found to reverse all of the GA₃-promoted changes in protein synthesis or mRNA levels in barley aleurone, whether they be increases or decreases¹. The results of the present study add to that list. ABA not only prevents the GA₃-promoted accumulation of α -amylase gene transcripts and of pHV14 transcripts in nuclei, but also prevents the suppression of total transcripts and of ribosomal transcripts by GA₃. Thus, the action of ABA leads to both promotion and inhibition of transcription and in this respect resembles GA₃. In summary, these experiments indicate that the inhibitory effect of ABA on α -amylase synthesis and on the accumulation of α -amylase mRNA²⁻⁹ can be accounted for largely by its effects within the nucleus, probably on gene transcription. A cytoplasmic role for ABA, in particular at the level of translation¹, in the conditions of these experiments would appear to be unnecessary.

These results also exemplify the complexity of GA₃ action. GA₃ has multiple effects in cereal aleurone, increasing the concentration of some mRNAs and proteins and decreasing the concentration of others^{5,18}. Here against a background of reduced total transcripts, GA₃ caused suppression of ribosomal gene expression and an increased expression of two other genes (Fig. 2). It seems, therefore, that the multiplicity of effects elicited by GA₃ on protein and RNA levels is also seen at the level of transcription.

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Cooperative dimeric and tetrameric clam haemoglobins are novel assemblages of myoglobin folds

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Cooperative functioning of many protein systems depends on communication between different subunits of those systems. Perhaps the best understood cooperative protein system is the vertebrate haemoglobin tetramer, in which the subunits share a similar tertiary structure (the myoglobin fold) with each other and with myoglobins and haemoglobins from at least four different animal phyla and leguminous plants. Blood clams have cooperative tetrameric haemoglobin and, in addition, a cooperative homodimeric haemoglobin¹⁻⁶. In view of previous reports^{7,8} concerning the role of dimers in the vertebrate tetramer, the clam haemoglobins represent a very interesting model system. We report here the low-resolution three-dimensional crystal structures of the dimeric and tetrameric cooperative haemoglobins from the blood clam *Scapharca inaequivalvis*. We find that clam haemoglobins are made of myoglobin-like subunits but their assembly to form dimers and tetramers is quite different from that of vertebrate haemoglobin. The arrangement of the subunits provides a simple structural explanation for haem-haem interaction in the dimer and tetramer.

The haemoglobins in *S. inaequivalvis* are formed from three distinct polypeptide chains, two of which associate to form heterotetramers (A₂B₂), while a third associates to form homodimers⁵. The dimers and tetramers bind oxygen cooperatively with Hill coefficients of 1.5 and 2.1, respectively⁶. The dimer shows no oxygen-linked subunit association phenomena, whereas the tetramer can associate to form higher polymers in the deoxy state⁵. Although complete amino-acid sequences are not available for the haemoglobins from *S. inaequivalvis*, the sequences of two haemoglobin chains from related clams have been determined^{9,10}; these sequences align functionally with sequences from other haemoglobins that possess the myoglobin

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Fig. 1 Composite sections from the 5.5-Å clam tetramer map (*A, B, D, E*), with equivalent sections from a 5.5-Å map of the α -chain of human CO haemoglobin (*C, F*). The Fourier coefficients for the map of the human α -chain were calculated from atomic coordinates obtained from the Protein Data Bank¹⁸ with an overall *B* value of 20 Å². *A, B*, the haem and E and F helices of the two distinct subunits in the clam tetramer map; *C*, the equivalent region from the structure factor map of the human α -chain. These density 'slabs' cover four sections or ~ 5.5 Å perpendicular to the plane of the paper. *D, E*, the G and H helices of the two distinct subunits in the clam tetramer map; *F*, those of the human α -chain. These density slabs cover six sections or ~ 9.2 Å perpendicular to the plane of the paper. Note particularly the additional electron-dense rod in the upper portion of *D* and *E* corresponding to the pre-A helix which is present in clam haemoglobin but not in other haemoglobins. The G helix density shown in *E* is the poorest density obtained for a helix in the tetramer map. (The G helix is on the outside of the molecule in the clam tetrameric haemoglobin).

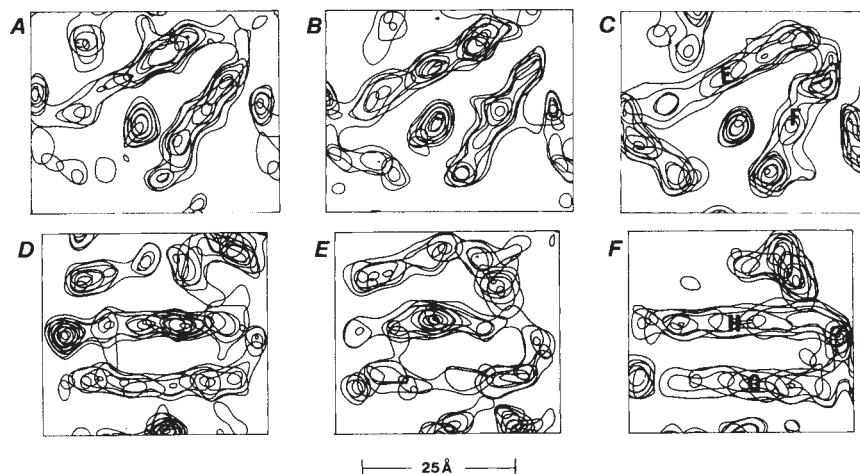
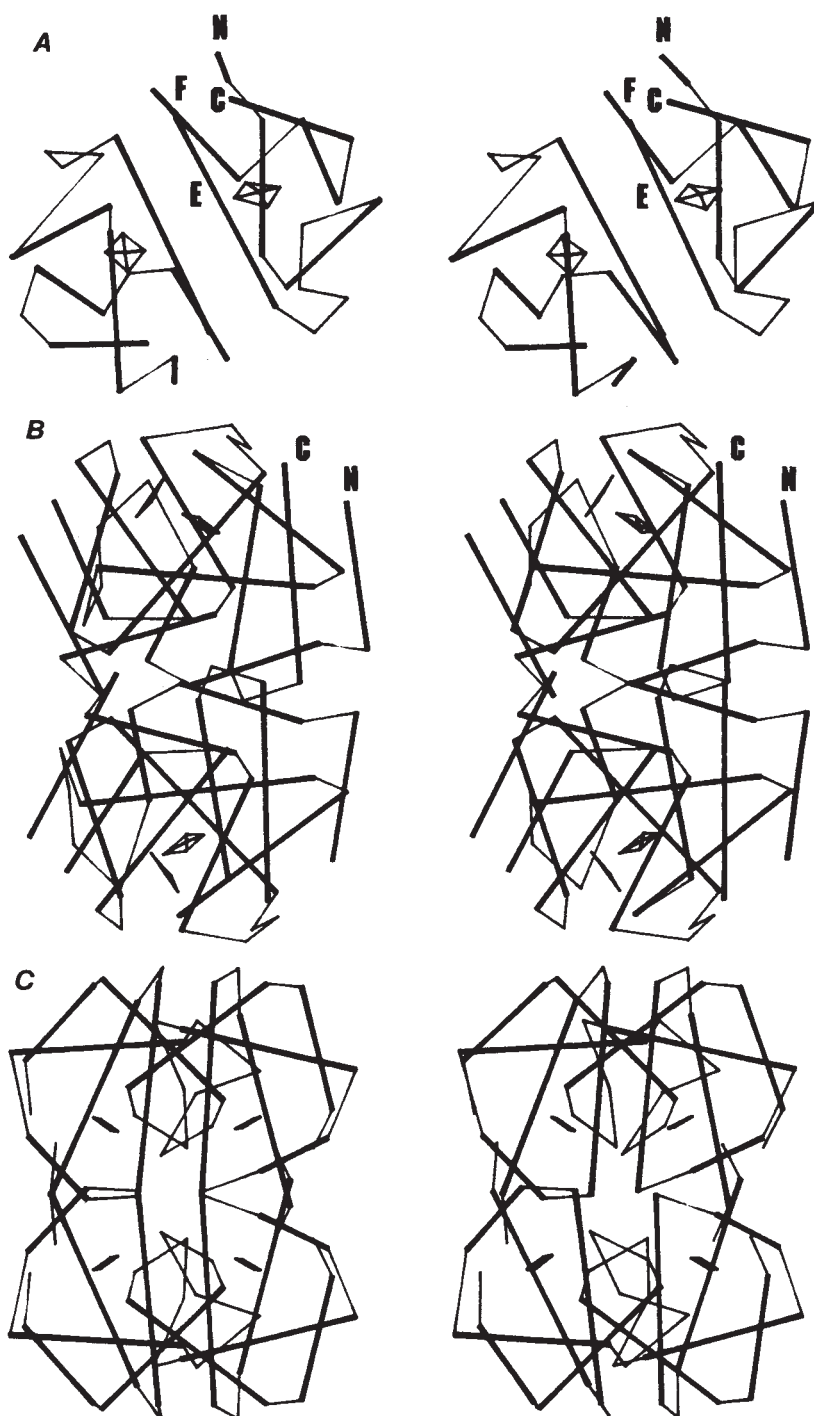


Fig. 2 Stereo diagrams showing arrangement of subunits in the clam haemoglobin dimer (*A*), clam haemoglobin tetramer (*B*) and human haemoglobin tetramer (*C*). The pre-A, A, B, E, F, G and H helices are drawn with heavy lines. The diagrams for the clam haemoglobins were drawn using helix end points taken from the tetramer map. The human CO haemoglobin diagram uses the α -carbon positions of residues at the end of helices, obtained from the Protein Data Bank coordinates. One subunit in both the clam dimer and tetramer is labelled N at the amino terminus and C at the carboxy terminus. *A*, clam dimer with the dyad relating the two subunits oriented perpendicular to the plane of the paper. Note how the E and F helices of the two subunits lie across each other to form an extensive contact. (The E and F helices are labelled in one subunit.) The slightly different chain path for the two subunits in the homodimer diagram is the result of the molecular replacement model and does not represent any real difference between the subunits. *B*, clam tetramer with the molecular dyad (used in symmetry averaging) oriented horizontally in the plane of the paper and the pseudo-dyad relating the two subunits within a dimer, also approximately in the plane of the paper. *C*, human CO haemoglobin tetramer with the molecular dyad lying horizontally in the plane of the paper and the pseudo-dyad relating α_1 to β_1 lying vertically in the plane of the paper. In vertebrate haemoglobins the pseudo-dyad is perpendicular to the molecular dyad and together they generate a third dyad. Thus, vertebrate haemoglobin has approximate 222 point group symmetry. In the clam tetramer these dyads are not perpendicular, a third perpendicular dyad is not generated, and thus the clam tetramer does not have approximate 222 point group symmetry.



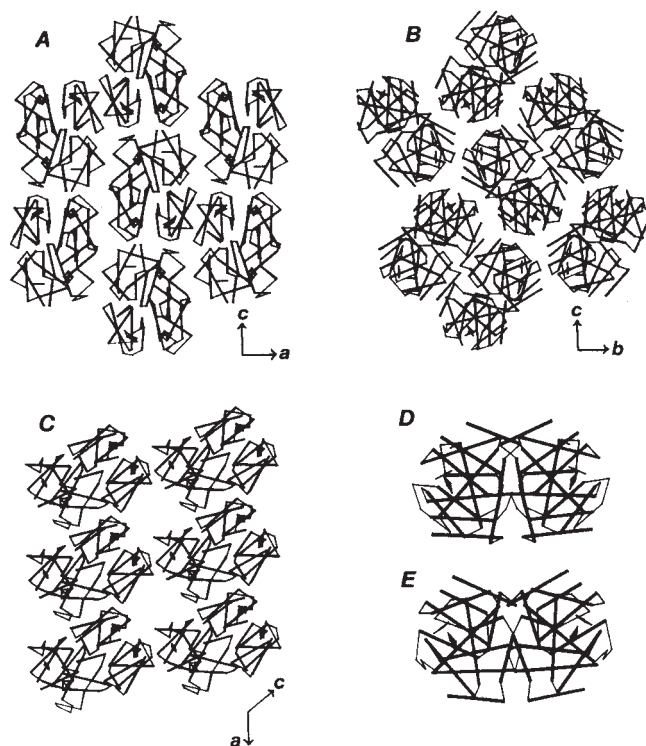


Fig. 3 The packing of dimeric and tetrameric clam haemoglobins in their crystals. Line drawings of the subunits as in Fig. 2. *A*, seven tetramers projected down *b*; the most extensive contact is between the central tetramer and the one at the lower right. *B*, seven tetramers projected down *a*. A fairly extensive contact can be seen between the central tetramer and that at the upper right, which is related to it by a crystallographic dyad axis. The space group symmetry and packing considerations are incompatible with these contacts being intramolecular. *C*, twelve dimers projected down an axis 30° away from *b*. The *b* axis is oriented into the paper, to the right. The tight association occurs across the crystallographic dyad axis along *b*. *D*, close-up of two dimers related by the crystallographic dyad along *b*, which is vertical in the figure. *E*, close-up of the tetramer with the molecular dyad vertical. Note that the dimers associate in dimer crystals much as do the half-molecules in the tetramer, but the two dimers are slightly less tightly packed than are half-molecules in the tetramer. The distances between iron atoms in neighbouring dimers are 47.5–52.5 Å, whereas the corresponding distances in the tetramer are 45.5–49.1 Å.

fold, and, in common with the hagfish, lamprey and shark, they have additional residues at the amino terminus.

The haemoglobin used in the present study was provided by Dr E. Chiancone (University of Rome). Table 1 shows data on crystal preparation and lattice constants for both dimeric and tetrameric carbon monoxide haemoglobins. Both types of crystal are robust in the X-ray beam and diffract well past 3 Å resolution.

A 5.5-Å electron density map of the tetramer crystals was calculated using phases obtained from isomorphous and anomalous differences for uranyl acetate and gold cyanide derivatives and from isomorphous differences for a potassium *cis*-dichlorodiamine platinate derivative. The 'phasing power' ($\langle f_H \rangle / \langle \text{error of closure} \rangle$) for the uranium, gold and platinum derivatives was 2.2, 1.6 and 1.1, respectively. The mean figure of merit was 0.79. This map contained many electron-dense rods, presumably corresponding to α -helices. The probable molecular envelope was established by considering the relatively empty solvent regions in combination with the packing constraints imposed by the location of the symmetry elements of the space group. Tentative iron positions, which subsequently proved to be correct, were identified by high electron density and small but consistent peaks in anomalous difference Fourier

Table 1 Dimeric and tetrameric crystal growth and lattice data*

	Buffer	pH	Space group	<i>a</i>	<i>b</i>	<i>c</i>	β	<i>z</i>
Dimer	2.5 M PO ₄	7.5	C2	93.3	44.1	83.6	122.0	1
Tetramer	2.4 M PO ₄	5.8	C22 ₂₁	102.1	94.1	128.0	–	1

* *z*, The number of molecules per asymmetric unit; *a*, *b* and *c*, Å; β , degrees.

maps for both the gold and uranium derivatives. Perhaps most importantly, we found a non-crystallographic molecular dyad relating the two gold sites, two minor uranium sites and most of the electron density in its neighbourhood. Eleven cycles of symmetry averaging about this dyad dramatically improved the quality of the map. The *R*-value for the final symmetry-averaged map was 21.4% ($R = \sum |F_0 - F_c| / \sum F_0$). The details of the determination of the tetramer and dimer structures will be published elsewhere.

In the final symmetry-averaged map, four myoglobin-fold subunits are evident. Each polypeptide chain can be followed with, at most, three short breaks. Each subunit contains an ellipsoid of very high density between the E and F helices, which corresponds to the haem group (see Fig. 1). An additional electron-dense rod ~18 Å long extends from the end of the A helix to the amino terminus of each chain; we believe this to be a helix and call it 'pre-A'. The pre-A helix is roughly parallel to, and in the same plane as, the G and H helices (see Fig. 1). It runs between the E–F corner and the H helix, and, in all subunits, the amino terminus is within 10 Å of the carboxy terminus. In retrospect, the pre-A helix made interpretation of the initial electron density maps difficult because in no other myoglobin folds do subunits have three roughly parallel helices. Other than the pre-A helix, the subunits have standard myoglobin folds.

The most striking feature of the clam tetramer structure is the manner in which the subunits are assembled. In vertebrate tetrameric haemoglobins, the G and H helices are on the inside of the molecule and are involved in subunit interactions, whereas the E and F helices are on the outside of the molecule and are exposed to solvent. However, in the clam haemoglobin tetramer, all the G and H helices are on the outside of the molecule and are exposed to solvent, whereas the E and F helices are involved in subunit interactions. Thus, the clam tetramer is 'back-to-front' relative to the vertebrate haemoglobins (see Fig. 2). The tetramer is a dimer of dimers with one dimer related to the other by the molecular dyad mentioned above. Within a dimer, the E–haem–F surfaces of the subunits face each other, forming an extensive contact.

The dimer contact involving the F helix invites speculation about the molecular basis of the cooperativity. In the deoxy form a contact similar to that seen in the liganded structure may clamp the subunits in a low-affinity state. In human haemoglobin, the F helix is displaced ~1 Å on CO binding¹¹, while in sperm whale myoglobin, the F helix is displaced by ~0.3 Å on oxygen binding¹². If displacements of the F helix occur in the clam haemoglobin subunits, binding of oxygen to one subunit would disrupt the deoxy contacts and allow the molecule to 'relax' to a partially liganded structure in which the unliganded haems have a higher affinity than those clamped in the deoxy structure.

Before the determination of the tetramer structure, we had little success with the dimer crystals. Lack of sufficient dimer crystals limited our heavy-atom search and we did not obtain any satisfactory molecular replacement solutions using various search molecules. However, on observing the back-to-front mode of association of the dimers in the tetramer structure, we speculated that the cooperative homodimer might be assembled similarly. A dimer constructed in this way could be cooperative, and from a balsa-wood model it appeared that the contacts were sufficiently extensive to be stable. In addition, a comparison of the sequences of a tetramer chain from *Anadara trapezia*⁹ with those of the dimer chain from *Anadara broughtonii*¹⁰ shows that although only 42% of the residues are identical in both chains,

84% of the residues in the E and F helices are identical. This result implies that the residues in the E and F helices are conserved between the tetramer and dimer chains for some important purpose such as contact formation.

Thus, we decided to use the dimer electron density from our tetramer map as a search molecule in molecular replacement. Structure factors obtained from the inverse Fourier transform of the isolated density were used in rotation¹³ and translation¹⁴ function calculations against the dimer data. Unlike our previous molecular replacement calculations, these gave good solutions.

We tested the validity of this molecular replacement solution using various criteria. First, the *R*-value of 42.8% compares favourably with other successful solutions^{15,16}. Second, dimer placement in the crystal showed no interpenetration between neighbouring molecules even though packing considerations were not included in the translation function (see Fig. 3). In addition, standard difference and 'haem-less'¹⁵ difference Fourier maps were calculated, examined and found to support this solution. Finally, an anomalous difference Patterson¹⁷ for native dimer crystals contained peaks at all positions for the expected iron-iron vectors.

The 'cooperative dimer' hypothesis of Antonini⁷ and colleagues in the late 1960s has been shown to be inapplicable to vertebrate haemoglobin⁸, in which the uncooperative $\alpha_1\beta_1$ dimer appears to move as an essentially rigid body. However, dimeric clam haemoglobin is cooperative, and a probable structural basis for that cooperativity has been revealed in the crystal structure. Because the same mode of subunit assembly occurs in the tetramer, it is likely that Antonini's cooperative dimer is

the underlying cause of cooperativity also in tetrameric clam haemoglobin.

To ascertain how these structures change with the release of ligands and why the dimers do not form tetramers in solution, we intend to determine the structures of liganded and deoxy dimeric and tetrameric clam haemoglobins at high resolution.

We thank Drs Emilia Chiancone and Eraldo Antonini for drawing our attention to these interesting proteins and for providing us with purified dimeric and tetrameric haemoglobins; and Drs Janet Smith and Wayne Hendrickson for the symmetry-averaging programs and many helpful discussions. This work was supported by NIH grant AM 02528. W.E.R. was supported in part by NIH training grant GM 07231.

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MATTERS ARISING

A complex of copper (II)-montmorillonite with a modified cyclodextrin

IN the article by Kijima *et al.*¹, structural organization of intercalated mono-(6- β -aminoethylamino - 6 - deoxy)- β -cyclodextrin (CDen) has been deduced from the interpretation of electron paramagnetic resonance (EPR) spectra of oriented film samples (CDen-Cu(II)-montmorillonite).

However, the invariance of the EPR spectrum towards the variation of the angle between the film plane and magnetic field is indicative of a random orientation of the adsorbed Cu(II) complexes rather than a spatial organization. For both models proposed in Fig. 3a and b, there should be a pronounced angular dependence of the spectrum with a maximum change at 45° in 'structure a' and a vanishing component at 90° in 'structure b'.

Since the statements in this article are inferred from an erroneous spectrum interpretation, the conclusions about the lipid bilayer orientation remain questionable.

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KIJIMA *ET AL.* REPLY—We admit that we have misinterpreted the electron spin resonance (ESR) spectra given in Fig. 2 of our paper. It is true that these spectra are indicative of a random orientation of the adsorbed Cu(II) complexes rather than a spatial organization, as pointed out by Motschi. This indication, however, appears impossible in view of the stereochemistry of hydrated Cu(II) ions in Cu(II)-montmorillonite¹. By using X-ray diffraction and scanning electron microscopy, we have re-examined the orientation of silicate layers in the film of CDen-Cu-montmorillonite used for ESR measurements. This re-examination leads us to conclude that the ESR spectra in Fig. 2 should be for a randomly oriented sample, not for an oriented film.

In considering the arrangement of intercalated CDen, ESR observations were used to justify two assumptions: (1) that the interlayer Cu(II) ion forms a coordination group [Cu(II) (*en*) (H₂O)₂]²⁺ with a square configuration and (2) that this square plane is perpendicular to the silicate layers. The correct data support assumption (1), but are inefficient in proving the other. However, it does not mean that the structure model proposed is invalidated. This is because it seems reasonable to adopt the second assumption, and because the structure model was fundamentally deduced from the interpretation of data for the interlayer spacing

and chemical composition of CDen-Cu-montmorillonite. Thus, our conclusions about the lipid bilayer orientation remain unchanged.

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